THE BASAL GANGLIA AS A STRUCTURE OF VOCAL SENSORY-MOTOR INTEGRATION AND MODULATION OF VOCAL PLASTICITY IN MAMMALS: BEHAVIORAL AND EXPERIMENTAL EVIDENCE FROM *Tadarida brasiliensis*

A Dissertation

by

JEDEDIAH TIM TRESSLER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2010

Major Subject: Zoology

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Approved by:

Chair of Committee,	Michael Smotherman
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ABSTRACT

The Basal Ganglia as a Structure of Vocal Sensory-Motor Integration and Modulation of Vocal Plasticity in Mammals: Behavioral and Experimental Evidence from *Tadarida brasiliensis*. (December 2010)

Jedediah Tim Tressler, B.S., West Virginia University

Chair of Advisory Committee: Dr. Michael Smotherman

The neural mechanisms underlying vocal motor control are poorly understood in mammalian systems. Particularly lacking are details pertaining to the mechanisms and neuroanatomical basis of sensory-motor integration and vocal plasticity, both of which are thought to be essential for evolutionarily advanced vocal behaviors like birdsong or human speech. Based on clinical evidence and imaging studies in humans, as well as its known significance for motor control in general, the basal ganglia (BG) have been hypothesized as a key site for audio-vocal integration, but direct evidence of this is lacking.

In this dissertation, I will fill this gap by providing experimental evidence that the basal ganglia are an important component of the forebrain vocal motor pathway. First, I present two examples of vocal plasticity in *Tadarida brasiliensis* that can serve as powerful behavioral assays of audio-vocal integration. Secondly I provide evidence of BG functions in audio-vocal integration by knocking down striatal dopamine levels with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrridine (MPTP). Finally, I will

utilize the D1-type receptor specific agonist SKF82958 and antagonist SCH23390 to examine how the direct pathway of the BG regulates vocal production and sensory-motor integration.

The behavioral results of these experiments indicate that the bats have a complex and context depended vocal response to noise stimuli that can be used to examine the neurological control of vocal plasticity. Further, the pharmacological evidence demonstrated that the BG was necessary for maintaining and modulating normal muscle force during vocal production. Finally, the mechanism of action in the basal ganglia was found to depend at least partly on activity at D1-type dopamine receptors.

The results of this dissertation support the hypothesis that the BG is a critical structure in the modulation of vocal commands in the forebrain vocal-motor pathway. Pathological or pharmacological disruption of dopamine signaling severely degraded the bats abilities to produce natural sounding calls or make adaptive changes to the acoustic environment. These results have implications for research into the treatment of basal ganglia disorders such as Parkinson's disease, providing an animal model for the study of hypokinetic dysarthria.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr, Smotherman, and my committee members, Dr. Garcia, Dr. Zoran, and Dr. Grau for their continuous support and guidance. Thanks also to Christine Schwartz, Jenna Jarvis, Dr. Bohn, and the rest of my many friends and colleagues in the Department of Biology for their ready assistance and fellowship. I would also like to express my appreciation to Dr. Garbutt for his inspiration and understanding. Finally, I would like to extend my gratitude to the NIH whose funding made this research possible.

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CHAPTER I

INTRODUCTION

Vocalization requires the complex coordination of multiple respiratory, laryngeal and supralaryngeal motor units. The neural mechanisms underlying vocal motor control are poorly understood in mammalian systems. Two distinct neural pathways are thought to be involved in the production and modulation of mammalian vocalizations. Firstly, a so-called "visceromotor" pathway based on the cortical and limbic activation of midbrain vocal pattern generators is found in all mammals and represents a phylogenetically old pathway of vocal motor control in which several interconnected midbrain structures coordinate the activity of vocal motor neurons (Jürgens, 2002a). Secondly, in a subset of mammals possessing some evidence of vocal plasticity (a group currently limited to primates, cetaceans and bats) an extrapyramidal forebrain pathway is suspected of providing a neuroanatomical basis for context- and sensory-feedback dependent modulation of vocalizations (Jürgens, 2009). This second pathway is of considerable interest because there are reasons to believe it played an important role in the evolution of human speech and language (Jarvis, 2004). The hallmark of human speech is its extraordinary plasticity, which in turn provides a basis for a seemingly endless increase in complexity. No other mammal's vocal behavior comes close to the complexity and sophistication of human speech, but a few animals, especially whales, dolphins and bats, are in fact capable of unusually complex vocal behaviors such as

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This dissertation follows the style of the Journal of Comparative Physiology A.

singing and vocal learning. Not coincidentally, cetaceans and bats both rely upon a highly plastic vocal behavior known as echolocation, and it is plausible that enhanced vocal plasticity supporting echolocation also underlies the expanded vocal communication repertoires in these animals. In other motor systems this type of plasticity is attributed to basal ganglia functions, yet currently there is no direct evidence linking an extrapyramidal pathway through the basal ganglia to vocal plasticity in any mammal other than humans. This dissertation is intended to fill this gap in knowledge.

Understanding how the vocal-motor circuits are functionally arranged would provide an invaluable advancement in our understanding of the evolution and control of mammalian vocalization. Since sensory feedback drives all examples of vocal plasticity in mammals, an important part of understanding the functional organization of the mammalian vocal motor pathways is to determine where in the vocal motor pathway auditory sensory feedback is incorporated. The basal ganglia have emerged as a likely candidate for the site of sensory-motor integration in the forebrain vocal control pathway, and within the basal ganglia, the neurotransmitter dopamine plays a critical role in regulating activity in the motor control pathways. The experiments outlined in this thesis are designed to 1) characterize how sensory feedback influences vocalizing, and then 2) establish a role for the basal ganglia in vocalizing by demonstrating how the pathological loss of dopamine (DA) influences sensorimotor integration in vocal control in the Mexican free-tailed bat (*Tadarida brasiliensis*). By examining the role of DA in sensory-motor integration, this study hopes to provide greater understanding of the role of the BG in vocal-motor control.

Mammalian vocal-motor pathway

In the midbrain pathway, the vocal component of the periaqueductal grey (PAG) receives descending inputs from the anterior cingulate cortex (ACC), the amygdala, the superior colliculus (SC) (Jürgens and von Cramon, 1982; Dujardin and Jurgens, 2005); and possibly the substantia nigra (Sinha and Moss, 2007). Projections from the PAG synapse with the nucleus ambiguus (NA) (Mantyh, 1983) and parabrachial nucleus (PB) (Mantyh, 1983; Krout et al., 1998) which control activity of the laryngeal (Schweizer et al., 1981) and respiratory motor neurons (Saper and Loewy, 1980) respectively. Projections also synapse extensively within the reticular formation (RtF) (Mantyh, 1983; Hannig and Jurgens, 2006) and nucleus retroambiguus (NRA) (Zhang et al. 1995 (Zhang et al., 1995; Vanderhorst et al., 2000). The NA and RtF are further interconnected with the PB, NA, as well as each other (Thoms and Jürgens, 1987; Holstege, 1989; Vanderhorst et al., 2000) forming the basis of a complex motor pattern generator. (See Fig. 1.1)

In the extrapyramidal pathway, the laryngeal area of the motor cortex exerts direct control of activation of the midbrain vocal pattern generators via projections to the RtF (Simonyan and Jurgens, 2003), bypassing the ACC and PAG. The motocortical larynx area also projects to the basal ganglia (BG). The BG projects to the ventrolateral thalamus, which in turn feeds back to the motor cortex (Jürgens, 2002b). (See Fig. 1.1) It is hypothesized that the cortical-striatal-thalamic loop preprocesses commands sent by the motor cortex to the RtF (Jürgens, 2009).

The basal ganglia

The basal ganglia (BG) are a complex subcortical forebrain structure that is known to play an important role in motor control. The BG functions to promote desirable motor patterns and suppress inappropriate ones, as well as alter motor patterns to match contextual needs. Several lines of evidence from studies of birdsong and human speech (Doupe and Kuhl, 1999; Kuhl, 2003) have suggested that the BG also plays an important role in the control of vocalization. Pathological dysfunctions of the BG are also suspected of causing or contributing to many of the most common human speech motor disorders (Alm, 2004).Understanding what role the BG plays in the production of vocalization would greatly increase our knowledge of how the mammalian vocal control pathway works, particularly for volitional or learned vocalizations.

Basal ganglia general structure and function

The BG is comprised of 5 discrete brain structures, the striatum (the caudate and putamen), pallidum, subthalamic nucleus and substantia nigra which are themselves comprised of subnuclei (Groenewegen, 2003). The primary input center to the basal ganglia, the striatum, receives excitatory inputs from the cerebral cortex, midline, intraliminar thalamic nuclei, the hippocampus and amygdala (Parent and Hazrati, 1995). Connections from the cortex to the striatum are arranged topographically (Parent and Hazrati, 1995). The action of the striatum is regulated by the activity of dopamine releasing cells originating in the substantia nigra pars compacta (SNc). Two parallel output pathways lead from the striatum to the thalamus (Hikosaka, 1991; Nambu, 2004; Grillner et al., 2005). In the so-called, direct pathway, inhibitory projections from the

striatum, specifically the putamen, synapses in the substantia nigra pars reticulata, which sends projections to the thalamus. In the indirect pathway, inhibitory projections from the striatum synapse in the internal and external segments of the globus pallidus (GPi and GPe respectively) of the pallidum. The GPi directly inhibits activity in the thalamus. The GPe, however sends inhibitory projections to the subthalamic nucleus (STN), the STN sends excitatory projections to the SNr, and the SNr to the thalamus. The connections from the SNr and the thalamus are inhibitory. (See figure 1.1) Ultimately, the thalamus connects with the motor cortex, and it is through these connections that the BG influence motor commands.

The major output pathways from the BG display are tonically active and inhibitory. Desirable motor commands are ultimately facilitated by dis-inhibition of the thalamocortical projections. In a manner that is not completely understood, inputs from sensory, memory, and emotional centers alter the activity of dopaminergic neurons. Increased release of dopamine (DA) in the putamen results in increased activity in the direct pathway, while simultaneously decreasing activity in the indirect pathway. Activation of the direct pathway results in suppression of the tonic inhibition from the SNr to the thalamus. Activation of the indirect pathway, facilitated by low striatal DA levels, increases the inhibitory output of the SNr and GPi. Thus, striatal DA levels heavily influence BG activity (Mink, 1996).

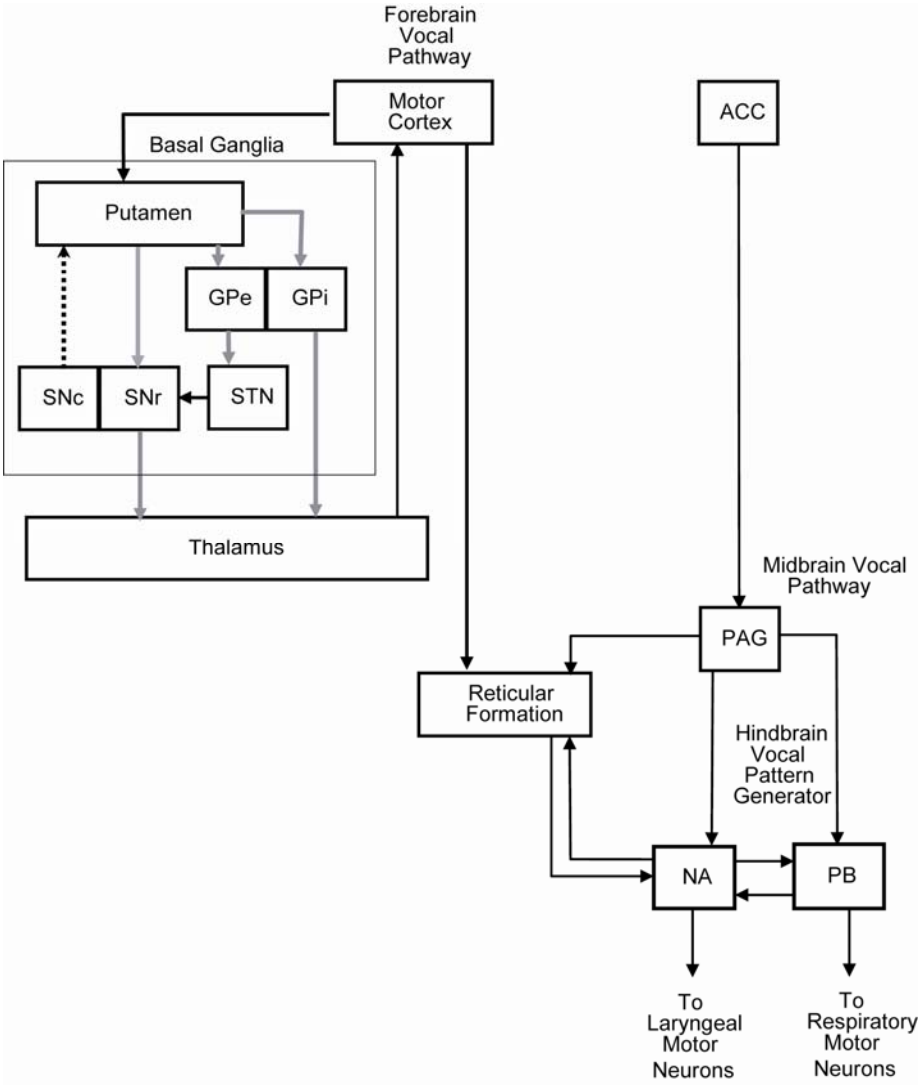

Fig 1.1. The major components of the mammalian vocal-control pathways. The major components and connections of the vocal control pathways are shown above schematically. Additionally, the primary structures and connections in the basal ganglia motor control loop are shown. For this section only, solid black arrows indicate an excitatory connection, and solid grey represents inhibitory. The dashed line represents the projections of dopamine producing cells.

Evidence for a role of the basal ganglia in vocalization

The initial evidence that the basal ganglia was involved in the control of vocalization was from the symptoms of accidental lesions (for review see (Jürgens, 2002b), and Parkinson's disease in humans. In general, lesions of the BG structures does not eliminate the ability to vocalize, but rather alters the ability of the subject to correctly control and alter volume and pitch (Groswasser et al., 1988).

Parkinson's disease is a debilitating mental illness caused by a decrease in levels of striatal dopamine as the result of the death of dopamine producing cells in the substantia nigra (Dauer and Przedborski, 2003). In addition to various motor deficits, a collection of speech disorders, known collectively as hypokinetic dysarthria, often accompanies the early onset of Parkinson's disease. Hypokinetic dysarthria is characterized by a harsh and breathy voice, a reduced voice bandwidth, reduced articulation, and reduced voice volume, also known as hypophonia (Sapir et al., 2008). These symptoms often appear in advance of sever motor deficits, and have a major impact on a patients quality of life in the early disease stages. Unfortunately, symptoms of hypokinetic dysarthria often prove resistant to or acerbated by treatment by traditional Parkinson's treatment techniques (Louis et al., 2001).

That the BG is involved in vocalization in humans has been further supported by fMRI studies. The putamen has shown increased activation in several aspects of vocalization (Brown et al., 2009), including when tasks involve sequence selection (Soros et al., 2006), and control of phonatory and articulatory apparatus (Bohland and Guenther, 2006). Unfortunately, while imaging studies can confirm that the BG is active

during certain vocal-motor tasks, they have been unable to suggest how they are regulating speech.

Until recently, it was believed that the BG was not involved in non-human mammal vocal control. However, resent experiments on the 50kHz ultrasonic vocalization in rats has shown that decreased dopamine levels leads to decreased call volume and bandwidth in rat ultrasonic mating calls (Ciucci et al., 2007; Ciucci et al., 2009). In songbirds, the role of the basal ganglia in song learning and adult vocal plasticity is now well established (Jarvis, 2004; Doupe et al., 2005; Kao et al., 2005). These results suggest that the basal ganglia may be an important part of vocal motor control for terrestrial vertebrates wherever vocal plasticity is an important aspect of the communication behavior.

Mammalian vocal responses to noise

Auditory stimulation has been found to influence vocalizing in just about every vertebrate studied. These effects fall into two main categories, 1) developmental (i.e. vocal learning) and 2) adaptive (for example temporary responses to background noise). Establishing where in the brain auditory information is incorporated into vocal motor pattern generation is an important step in understanding how vocal output is controlled. Examining how an animal responds to noise can provide important clues to the nature of auditory-vocal interactions.

Until recently, mammalian vocalizations have been characterized as genetically hard-wired and relatively insensitive to acoustic stimuli. However, research in primates,

cetaceans, and bats has begun to challenge this assumption. Primates have now been shown to exhibit several specialized vocal responses to calling in acoustically cluttered environments. Cotton-top tamarins exhibit a Lombard response similar to humans, and have been shown capable of anticipating intermittent bouts of white noise and both time calls to coincide with, and truncate calls to fit within bouts of intermittent silence (Egnor and Hauser, 2006; Egnor et al., 2007). Humans demonstrate a pitch shift reflex, in which a speaker unconsciously alters the fundamental frequency of there vocal output in response to hearing altered auditory feedback of their own voice (Burnett et al., 1997; Burnett et al., 1998). This reflex is being studied as a possible treatment for human vocal disorders (for example (Kalinowski et al., 1993; Stuart et al., 1997). Unfortunately, the neural mechanisms underlying either of these responses are not well understood.

Echolocating bats have a unique additional consideration for auditory interference compared to other terrestrial mammals. Not only are their vocalizations used for individual-to-individual communication, they are also reliant on their calls as a means of navigation and foraging (Schmidt and Joermann, 1986). As an organism utilizing an active sensory system, they would be particularly susceptible to acoustic interference, or jamming, and thus would likely develop a jamming avoidance response (JAR) like that seen in weekly electric fish. Characterizing the JAR of Mexican free-tailed bats provides important information on vocal plasticity, and provides a reliable and accurate behavior for exploring the neural mechanisms underlying vocal control and auditory feedback.

Presented experiments

This manuscript will present the results of three main experiments. In Chapter I1, the effects of two different kinds of acoustic stimuli, narrow and broadband noise, on *Tadarida brasiliensis* echolocation calls will be presented. The results from this experiment will serve as a basis for our pharmacological investigations of vocal motor control. In Chapter III, the neurotoxin 1-methyl-4-phenylpyridine (MPTP) will be used to induce a Parkinson's like state in order to examine how decreased levels of dopamine effect echolocation behavior, call structure and Lombard response. Finally, in Chapter IV, I will show that a drug targeting a specific dopamine receptor subtype found in the basal ganglia can reproduce some of the deficits caused by MPTP, which provides evidence for the role of the "direct" pathway through the basal ganglia specifically on the control of the Lombard response and echolocation call amplitude and duration.

CHAPTER II

CONTEXT-DEPENDENT EFFECTS OF NOISE ON ECHOLOCATION PULSE CHARACTERISTICS*

Introduction

A diverse range of animals alters the acoustic structure of their vocalizations in the presence of background noise. The nature and magnitude of these voice changes are interesting in both an ecological context (Brumm and Slabbekoom, 2005) and in a neurobiological context, particularly as it relates to the evolution of speech and language (Sinnot et al., 1975). Experiments with animals that can change the sound of their voice may offer insight into the neural basis of human speech. Early experiments found that noise stimuli caused monkeys to call more loudly (Sinnot et al., 1975), but since then vocal adaptations for calling in noise have been reported for many other animals, including frogs (Lopez et al., 1988; Penna et al., 2005), birds (Potash, 1972; Manabe et al., 1998; Cynx and Von Rad, 2001; Brumm and Todt, 2002; Pytte et al., 2003; Leonard and Horn, 2005), terrestrial and aquatic mammals (Sinnot et al., 1975; Nonaka et al., 1997; Brumm et al., 2004; Foote et al., 2004; Scheifele et al., 2005; Egnor and Hauser, 2006; Holt and Noren, 2009), and humans (Lombard, 1911). In every animal so far tested, vocalization amplitude was elevated by noise, syllable durations are often lengthened, and vocal pitch is sometimes elevated. This suite of changes in acoustic

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* With kind permission from Springer Science & Business Media: Journal of Comparative Physiology A, Context-Dependent Effects on Echolocation Pulse Characteristics, 195(10), 2009, 923-34, Tressler and Smotherman.

structure is likely to be biomechanically linked in most animals, driven principally by increases in call loudness (Lane and Tranel, 1971), and collectively may be viewed as generic adaptations that promote signal transmission in noise.

Echolocation behavior is particularly sensitive to the degrading effects of background noise, and animals that echolocate may be expected to display specialized vocal responses in addition to or in place of the generic vertebrate response to noise. The so-called "jamming avoidance response" (or JAR) exhibited by bats appears to be one such specialized behavior wherein the bats reportedly shift the frequency of their echolocation pulses (Ulanovsky et al., 2004; Gillam and McCracken, 2007; Gillam et al., 2007; Bates et al., 2008) to minimize overlap with interfering noises. Many previous studies have reported that the most effective stimulus for eliciting vocal changes were noise stimuli overlapping in frequency with an animal's own communication sounds (Sinnot et al., 1975; Schwartz and Wells, 1983; Brumm and Todt, 2002), and in several studies the vocal response to interfering noises included an elevation in vocal pitch (Lombard, 1911; Lane and Tranel, 1971; Van Summers et al., 1988; Nelson, 2000; Leonard and Horn, 2005). Field studies on free-tailed bats (*Tadarida brasiliensis*) reported that the JAR occurred in the absence of changes in amplitude (Ulanovsky et al., 2004; Gillam and McCracken, 2007; Gillam et al., 2007), but accurate measures of echolocation pulse amplitude are difficult to obtain in the field. These field studies also reported several other concurrent changes in pulse structure not directly associated with jamming avoidance, such as changes in duration and bandwidth, leaving open the possibility that the recorded changes in pulse pitch were byproducts of a more

generalized response to noise. It was also observed that the free-tailed bats showed a bias towards upward frequency shifts and that they rarely succeeded in completely avoiding overlap with the interfering noise (Ulanovsky et al., 2004; Gillam et al., 2007), which led researchers to speculate that the bats were minimizing overlap with only a restricted terminal portion of the pulse (Gillam et al., 2007). To test whether the free-tailed bats' JAR is indeed a context-dependent behavior that is distinguishable from the generic vertebrate response to noise, a thorough analysis of how they respond to broadband noises versus band-limited noises was required, and it needed to be done in the lab so that all pulse acoustic parameters including amplitude could be accurately accounted for.

The most common way animals improve the propagation of their communication signals in noise is by increasing signal amplitude. The human Lombard response (Lombard, 1911), characterized by increased voice amplitude in noise, has also been observed in frogs (Lopez et al., 1988; Penna et al., 2005), several species of birds (Potash, 1972; Manabe et al., 1998; Cynx and Von Rad, 2001; Brumm and Todt, 2002; Pytte et al., 2003; Leonard and Horn, 2005), and many mammals, including cats (Nonaka et al., 1997), whales (Foote et al., 2004; Scheifele et al., 2005; Holt and Noren, 2009) and primates (Sinnot et al., 1975; Brumm et al., 2004; Egnor and Hauser, 2006). Increasing pulse loudness carries with it some ecological disadvantages, including increased energy expenditure and the potential for attracting predators (Dabelsteen et al., 1988; Brumm and Todt, 2002). Thus, there are selective pressures favoring the use of other vocal adaptations. Animals may also compensate for noise-induced signal

degradation by increasing syllable duration (Lane and Tranel, 1971; Picheny et al., 1986; Brumm et al., 2004; Foote et al., 2004; Leonard and Horn, 2005; Penna et al., 2005; Egnor and Hauser, 2006), adjusting call timing (Brumm, 2006; Egnor et al., 2007), and shifting pitch (Ulanovsky et al., 2004; Gillam et al., 2007; Bates et al., 2008). Echolocating bats are known to tightly regulate the loudness of their echolocation pulses (Kobler et al., 1985; Hiryu et al., 2007) and they pulse louder in the presence of echolocating conspecifics (Schmidt and Joermann, 1986) and broadband noise (Simmons et al., 1978; Bates et al., 2008). Also, echolocating bats constantly adjust pulse durations to suit the range of the current target or background distances and in response to noise (Simmons and Grinnell, 1988). Big brown bats (*Eptesicus fuscus*) lengthened their pulses in broadband noise but did not lengthen their pulses in the presence of a tonal interfering stimulus (Bates et al., 2008).

Under natural conditions, bats routinely make stereotyped coordinated changes in multiple pulse parameters. In many species of bat, an inverse relationship exists between duration and bandwidth (Jones, 1999; Schnitzler and Kalko, 2001). As the coordination of these changes can be explained by both behavioral and mechanical coupling, the extent to which a bat can make changes in pulse intensity, duration, and frequency independent of one another is not yet clear. Elucidating the degree of coupling between pulse parameters would provide insight into how vocal pathways are controlled. The vocal response to noise could provide a tool for distinguishing individual parameter control if there were unique responses to different acoustic stimuli. Collectively such data would be useful if it shed light on how the underlying audio-vocal

neurocircuitry is organized. The extent to which different acoustic parameters of animal vocalizations are controlled independently is particularly important because this information would reflect upon the functional architecture of the vocal motor pathway, as well as the extent to which animals can exert control over the sound of their voice.

The JAR exhibited by free-tailed bats has so far only been studied in the field where long duration, narrow bandwidth search pulses were emitted (Ratcliffe et al., 2004; Ulanovsky et al., 2004; Gillam and McCracken, 2007; Gillam et al., 2007). In the laboratory free-tailed bats responded to background noise by emitting louder pulses with longer durations and greater bandwidths (Simmons et al., 1974), but their JAR behavior has not been examined in the lab. Since the pulses utilized in the lab have a broader bandwidth, emphasize higher frequencies, and are less than half as long as the average search pulse (Schwartz et al., 2007), it was possible to assess whether the vocal responses to noise would be dependent on the pulse type emitted and the frequency range of noise present. The pulse types used by free-tailed bats in the laboratory are identical to those used by free-tailed bats in the wild when flying in cluttered and confined spaces, such as in caves and other roost sites where many other bats are likely to be present. Results obtained in these studies are therefore directly applicable to the natural behavior of free flying bats at times when they are most likely to encounter acoustic interference. It was hypothesized that if the bats continued to perform JAR in the lab, the most effective frequency for evoking the JAR would be shifted upwards to correspond to the elevated frequencies emphasized by the bats in the laboratory. Alternatively, it was possible that the bats would exhibit a highly stereotyped vocal

response identical to the one observed in the field and exclusively dependent on interfering frequencies overlapping with outdoor search pulses rather than the pulses emitted in the lab, which would cast the JAR behavior as more of an inflexible acoustic reflex. The results of these studies indicate that the JAR behavior exhibited by free-tailed bats appears to be a flexible context-dependent vocal behavior and neither an extension of the generic vertebrate response to noise nor a simple acoustic reflex.

Methods

Animal husbandry

Twenty Mexican free-tailed bats, *Tadarida brasiliensis mexicana*, were caught wild from a year round roost on the campus of Texas A&M University and housed in the Texas A&M Department of Biology vivarium facility. Bats were kept on a phase-shifted 12/12 day/night cycle, with vivarium lights turning off at 12:00pm. The bat vivarium was a temperature and humidity controlled room that was large enough to allow the bats to fly freely. Bats were trained to feed themselves and had to fly daily to obtain food. The bats were fed a diet of mealworms supplemented with vitamins, minerals and essential fatty acids. All husbandry and experimental procedures were in accordance with NIH guidelines for experiments involving vertebrate animals and were approved by the local IACUC.

Acoustic stimuli

Acoustic stimuli consisting of either broadband or band-limited noise was generated digitally with Tucker-Davis Technology (TDT) system III hardware and the

openEX software v5.4. The broadband noise was digitally filtered to present a total signal bandwidth spanning a range of 15 to 100 kHz, which covered the entire range of the two loudest harmonic components of *Tadarida brasiliensis'* echolocation pulses. The band-limited noises were generated by digitally bandpass filtering white noise down to a bandwidth of 5 kHz. A five-kilohertz stimulus bandwidth was chosen based on pilot data indicating that this was the smallest bandwidth that reliably evoked consistent changes in pulse structure. Pure tones such as those used by Bates et. al., (2008) had no significant effects on pulse parameters. I tested a frequency range of five-kilohertz bandwidth signals that spanned from approximately 10 kHz below to 10 kHz above the average lowest and highest frequencies, respectively, of the principal harmonic component of the bat's echolocation pulses. These 5 kHz bandwidth stimuli were centered at either 17.5, 22.5, 27.5, 32.5, 37.5, 42.5, 47.5, 52.5 or 57.5kHz. All stimuli were played through a Sony amplifier (model # STR-DE598) driving a 4-speaker array composed of 2 Pioneer Ribbon Tweeters (ART-55D/301080) and 2 Pioneer Rifle Tweeters (ART-59F/301081), arranged to project across the flight path and in both directions along the length of the tunnel. Each speaker provided a flat (±3 dB) output at a maximum of 85 dB SPL across the principal frequency range of interest, roughly 15 to 60 kHz. In order to test the effects of broadband stimulus intensity on echolocation pulse structure, broadband noise amplification was reduced by 10, 20 and 30 dB relative to the maximum. The bats' echolocation pulses ranged in intensity from 80 to 115 dB SPL during flight, as measured by a microphone placed in the center of the room. All experiments were performed with flying bats rather than stationary bats because

echolocation pulses from flying bats were observed to be less variable than those emitted by stationary bats, and because it was assumed that flying bats would be more likely to exhibit a robust vocal response to noise than stationary bats.

All experiments were performed in an 8 meter long by 2 meter wide by 3 meter high flight tunnel lined with sound-absorbing 4-inch acoustic foam (Sonex ©,model UNX-4), with the lights off. Recordings of flying bats were made using a Bruel & Kjaer Free-field ¼" microphone (Type 4939) placed in the center of the room. The placement of the microphone was coordinated with the positions and directionality of the speakers to minimize the recorded intensity of the stimuli while maximizing the recorded intensity of the bat pulses, which facilitated the digital extraction of echolocation pulses from the background noise. Incoming signals were digitized with a National Instruments DAQmx, NI PCI-6251 (200 kHz, 16 bit sample rate), and viewed with Avisoft Recorder v3.0.

Echolocation pulse extraction and analysis

Recordings were analyzed using SASLab Pro v4.39. As the bat approached the microphone, only the last 10-15 pulses before the bat passed the vertical plane of the microphone were selected for analysis, ensuring that all analyzed pulses were emitted within approximately 1-meter depth of the microphone, and thus that pulses from the same relative time-period within the flight path were compared across all experimental conditions. Additionally, only pulses that were at least 15 dB louder than the recorded noise stimuli were included in the analysis to ensure accurate measurements of all acoustic parameters. I used the methods of Penna et. al. (2005) to subtract the

contributions of stimulus amplitude on the measurements of pulse amplitude. Doppler effects on the frequency of the echolocation pulses were accounted for in the post-hoc analysis.

In the flight tunnel, free-tailed bats emitted short (4-7 ms), downward frequency-modulated pulses that typically began around 45 kHz and ended around 25 kHz. The spectral parameters of the pulses were summarized by three measurements at three different time points within the pulse (Gillam and McCracken, 2007; Schwartz et al., 2007; Ulanovsky and Moss, 2007), including the frequency at the start of the pulse (F_{start}), the frequency of the end of the pulse (F_{end}), and the frequency of maximum pulse intensity (F_{peak}). F_{peak} was taken from the power spectrum, and F_{start} and F_{end} were defined as the frequencies at the lower and upper end of the spectrum –15dB relative to the intensity of the peak frequency (Schwartz et al., 2007; Surlykke and Moss, 2000). The slope of the pulse was calculated by subtracting the F_{start} from F_{end} and dividing by the duration of the pulse, providing a simple estimate of the overall rate of frequency change. One hundred echolocation pulses from each bat in each treatment were selected at random for analysis. For temporal analyses, I used 256-point fast Fourier transforms (FFTs) with 93.75% overlap, providing 976 Hz spectral and 0.064 ms temporal resolutions. For spectral analysis, 1024-point FFTs provided 244 Hz spectral and 0.256 ms temporal resolutions.

Experimental procedure

Twenty bats were chosen at random from the captive colony and randomly assigned to 2 groups of 10 bats each. Group 1 was used only for trials utilizing

broadband noise, group 2 only band-limited noise. The same individuals were not exposed to both stimuli to prevent a confounding effect from stimulus order and undue stress due to excessively long experimental trials. All experiments were conducted during the time of day when the bats were normally most active within the vivarium (10am-2pm). All individuals had previous experience flying in the chamber and had been habituated to the experimental procedures and daily handling. Bats were acclimatized to the experimental chamber before beginning each trial, and they were "warmed up" by letting them fly freely in the room before beginning experiments. For each trial, individuals were recorded flying back and forth multiple times between two perches located at opposite ends of the flight tunnel. Approximately 12 to 15 flights across the room were needed for each trial to collect the minimum number of pulses satisfying all of the threshold criteria defined above. Baseline recordings of bats flying back and forth between the perches in the absence of acoustic stimuli were recorded before beginning each experimental trial, and the various acoustic stimuli were presented in a pseudo-random fashion and were alternated with silent trials to track any potential changes in pulse parameters associated with time spent in flight.

Statistical analysis

All statistical procedures were performed utilizing SAS v9.2 and SAS-JMP v7.0.7. A MANOVA analysis was performed to determine if there was a significant effect of noise on echolocation pulse structure. If the effect of interference was shown to be significant by MANOVA ($P \leq 0.05$, $\alpha = 0.05$), the results of ANOVA analysis to determine significant effect within parameters was reported. Student's t-test pair-wise

multiple comparison procedure (α=0.05) was used to determine significant differences between different treatments within a parameter if a significant effect of noise was found. Both MANOVA and ANOVA analysis were conducted as a mixed model design with individual bat as a random variable. Results are given as means ± S.E., unless stated otherwise.

Results

Effects on broadband noise on pulse structure

The presence and intensity of the broadband noise had an effect on all pulse parameters (Fig. 2.1). As the intensity of the white noise increased, F_{start} and F_{end} increased and decreased respectively. F_{end} decreased significantly from baseline when the broadband noise was within 10 dB of maximum. F_{start} increased significantly for all stimulus intensity levels. Duration, bandwidth and pulse amplitude all increased with increased broadband noise loudness. All levels of stimulus loudness significantly increased both the duration and bandwidth from baseline levels. Pulse amplitude was not significantly affected by noise that was -30 dB of the maximum. Stimulus intensity had a significant effect on all pulse parameters except F_{peak}. Although F_{peak} appeared to be slightly elevated in the presence of noise, the change was not significantly different from baseline at any intensity (see Table 2.1 for numerical comparisons and p-values).

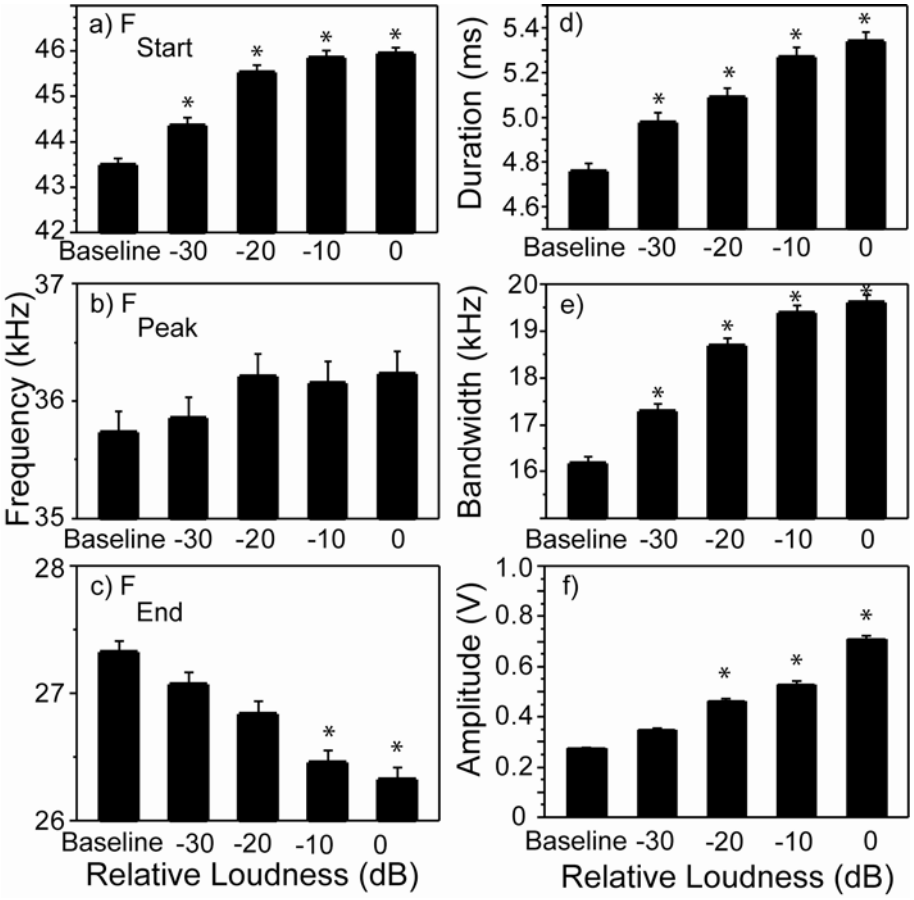

Fig. 2.1. The effects of broadband noises of varying intensity on echolocation pulse parameters. Each bar represents the mean of 10 bats flying in silence (baseline) or broadband noise. Zero dB represents the loudest broadband noise (85 dB-SPL), with magnitude decreased by 10, 20 and 30 dB from maximum. Error bars denote one standard error from the mean, asterisks designate results significantly different from baseline (α=0.05).

Table 2.1. The effect of broadband noise and band-limited noise. Centered at 32.5 kHz on the echolocation pulse parameters (mean ± std. dev.) of 2 groups of *Tadarida brasiliensis*. Column Δ gives the difference between noise off and on for each treatment. Values were obtained from 100 pulses per individual, 10 individuals per noise type. P-values are reported for ANOVA test of significance for noise on pulse parameter.

	Broadband Noise				**32.5 kHz Band-limited Noise**			
	Off	On	Δ	P-Value	Off	On	Δ	P-Value
Duration (ms)	4.76±1.19	5.34±1.31	0.58	<0.001	5.85±1.38	6.33±1.55	0.48	0.001
F_{start} (kHz)	43.50±4.93	45.95±4.36	2.45	<0.001	45.31±4.47	42.71±7.24	-2.60	<0.001
F_{end} (kHz)	27.33±2.94	26.33±2.94	-1.00	0.017	26.90±3.42	28.58±3.92	1.69	<0.001
F_{peak} (kHz)	35.74±5.88	36.24±6.01	0.49	0.965	37.85±5.63	39.56±5.52	1.71	0.002
Amplitude (V)	0.27 ±0.21	0.71 ±0.63	0.43	<0.001	0.45±0.27	0.59±0.42	0.13	0.357
Bandwidth (kHz)	16.17±4.97	19.62±4.81	3.45	<0.001	18.41±5.63	14.13±8.40	-4.29	<0.001
Slope (kHz/ms)	3.62 ±1.44	3.88 ±1.24	0.28	0.061	3.49±1.71	2.61±2.61	-0.88	<0.001

The distribution of F_{peak} was bimodal in both the presence and absence of broadband noise (Fig. 2.2); the two peaks in the histogram corresponded to two different pulse structures used by the bats while flying in cluttered spaces, one being a typical FM pulse with an F_{peak} near the center of the pulse, and the other being a quasi- CF-FM pulse wherein the bats include a short intense CF at the start of the pulse resulting in a higher F_{peak}. Broadband noise caused a slight change in the distribution of F_{peaks} that corresponded with an increase in the number of CF-FM pulses containing F_{peaks} between 40 and 45 kHz, however their was no statistically significant change in the mean F_{peak}. Since any observed changes in the mean F_{peak} could have been accounted for by either shifts in pulse frequency or switches in the relative numbers of pulse types emitted (FM versus CF-FM), the upper and lower modes were analyzed both separately and as a single population; however for broadband noises the statistical results were the same for F_{peak} in either case (i.e. no significant change in F_{peak}). These results were further confirmed by comparison of the distributions of F_{peak} between treatments. For all subsequent analyses of noise, stimuli on F_{peak} the relative contributions of changes in calling mode versus shifts in the median frequencies of each mode were taken into account and are presented where significant differences were observed.

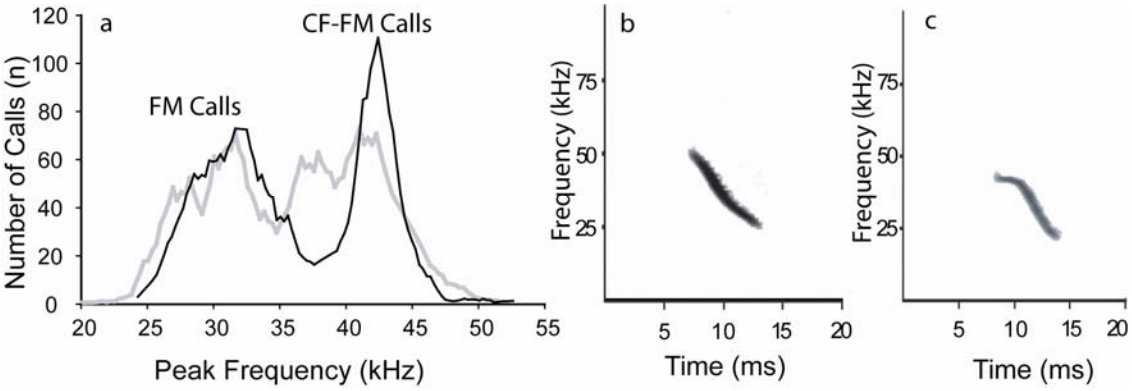

Fig. 2.2. Bimodal distribution of F_{peak} and representative echolocation pulse types. (**a**) The distribution of F_{peak} in the absence (grey line) and presence (black line) of broadband noise. Both curves represent the number of pulses (n) exhibiting an F_{peak} at the given frequency. The change in distribution did not result in a significant difference between silence and broadband trials. Spectrogram in **b** is representative of the FM type pulse, while the spectrogram in **c** is representative of the CF-FM type pulse.

Effects of band-limited noise on pulse structure

Inference had a significant effect on the F_{start} ($P<0.0001$) and F_{end} ($P<0.0001$). The effect of center frequency on the average F_{start} and F_{end} of the echolocation pulse can be seen in Figure 2.3, a&b. The response of F_{start} and F_{end} to band-limited noise stimuli differed from that observed for broadband noise. First, F_{start} significantly decreases from a mean baseline frequency of 45.3 +/- 4.4 kHz to 43.2 +/- 8.7 kHz at the band-limited noise of 22.5 kHz. The F_{start} remained at this lower frequency without significant change for the 27.5 and 32.5 kHz band-limited noise. For the 17.5 kHz band-limited noise and for all stimulus frequencies greater than 32.5 kHz, there was no significant change in F_{start} from the baseline. As seen in broadband noise, F_{end} initially decreased significantly from a baseline of 26.9 +/- 3.3 to 25.7 +/- 4.1 kHz at the 22.5 kHz band-limited noise. At the 32.5, 37.5 and 42.5 kHz band-limited noise, however, the F_{end} increased significantly above baseline to 28.6 +/- 3.9, 28.8 +/- 4.5, and 27.8 +/- 4.7 kHz respectively. At the 47.5 kHz band-limited noise the F_{end} decreases significantly from the 32.5 kHz band-limited noise but remained significantly greater than baseline frequency at 27.7 +/- 4.1 kHz. The F_{end} at the 17.5, 52.5, and 57.5 kHz band-limited noise were not significantly different from the baseline frequency.

The combined effect of the change in F_{start} and F_{end} resulted in a significant decrease in the bandwidth (Fig. 2.3,d; $P<0.0001$). In the presence of band-limited noise at 17.5 and 22.5 kHz I observed what appeared to be a decrease in echolocation bandwidth to 18.0 +/- 6.7 and 17.4 +/- 8.2 kHz but the change was not statistically significant. The 27.5 kHz band-limited noise did cause a significant decrease in

bandwidth down to 16.2 +/- 8.0 kHz. The maximum change in bandwidth was evoked by the 32.5 kHz band-limited noise, with a significant decrease to 14.3 +/- 8.3 kHz, which is opposite to the response observed when bats echolocated in the presence of broadband noise. As the noise center frequency was raised above the 32.5 kHz frequency, the bandwidth of the echolocation pulse increased to 16.0 +/- 7.8 kHz at 37.5 kHz band-limited noise and the bandwidth returned to a value not significantly different from baseline (17.2 +/- 6.7 kHz) at the 42.5 kHz band-limited noise.

A significant effect on duration in response to the band-limited noise was also observed (Fig. 2.3,c $P=0.0012$). As observed in response to broadband noise, I also observed an increase in duration for several band-limited noise. A significant increase from the 5.8 +/- 1.2 ms baseline pulse length to 6.3 +/- 1.4 ms occurred at the 22.5 kHz band-limited noise. Pulse duration was also significantly elevated by the 27.5 and 32.5 kHz band-limited noise. Pulse duration returned to within one standard deviation of the baseline duration for the 37.5 kHz band-limited noise and all stimuli at higher frequencies. Unlike the response to white noise however, the increases in pulse duration evoked by the band-limited noise were not accompanied by increased pulse amplitude. There was no significant effect of band-limited noise frequency on pulse amplitude (Fig. 2.3,f $P=0.3568$).

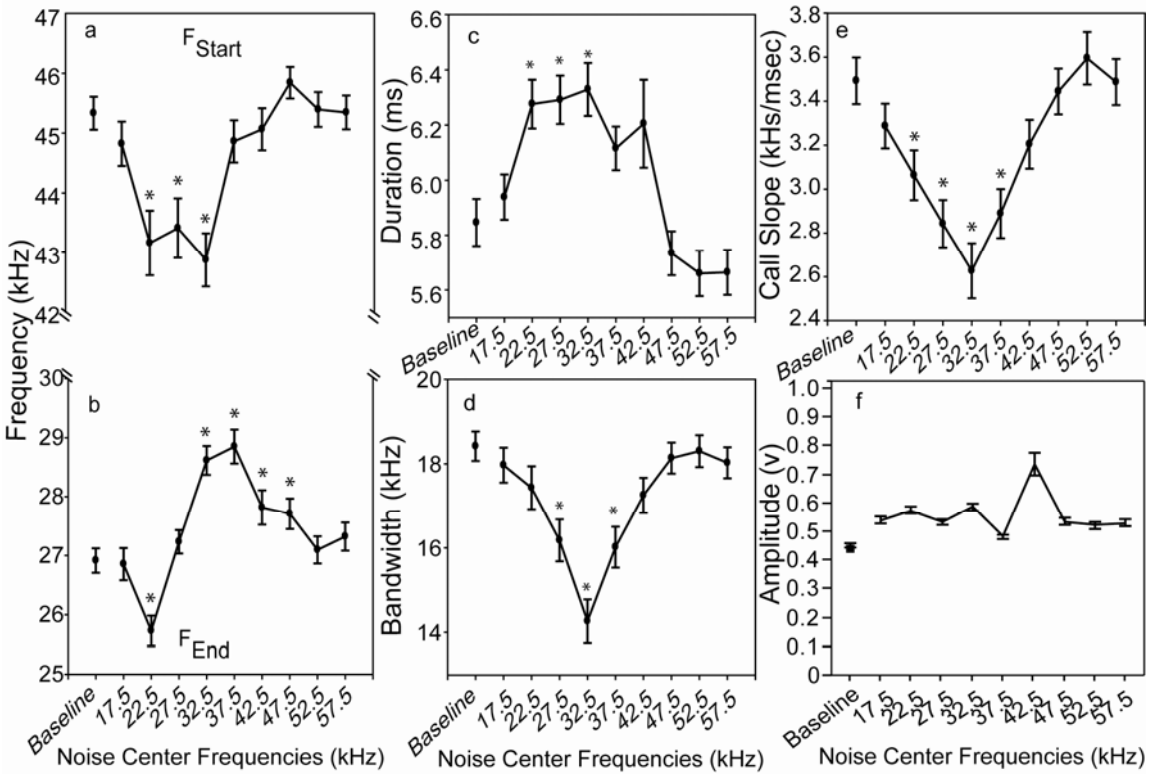

Fig. 2.3. Effect of the frequency of band-limited noise on echolocation pulse parameters. Baseline measurements were made in the absence of any noise stimulus. Each point represents the mean of 10 individuals, 100 pulses per individual. Error bars denote one standard error from the mean. There was no significant overall effect of band-limited noise on the amplitude of echolocation pulses (ANOVA P-value = 0.3568). Band-limited noise had a significant effect on all other parameters.

The decreasing bandwidth combined with the increasing pulse length resulted in a significant decrease in pulse slope (Fig. 2.3,e $P<0.0001$). In the absence of noise the average echolocation pulse had a slope of -3.5 +/- 1.7 kHz/ms. In the presence of the band-limited noise, the slope of the pulse decreased because the bandwidth decreased while the duration increased. The slope decreased linearly as the center frequency of the band-limited noise was increased from 22.5 kHz (-3.1 +/- 1.8 kHz/ms) up to 32.5 kHz (-2.6 +/- 2.0 kHz/ms),. Slope of the pulse then increased with increasing band-limited noise frequency until it returned to baseline levels at the 42.5 kHz band-limited noise and above.

Characterizing the effect of band-limited noise frequency range on F_{peak}

In contrast to the response to broadband noise, the F_{peak} changed significantly in response to the band-limited noise ($P=0.0016$) and the response pattern appeared slightly dependent on the stimulus frequency (Fig. 2.4). The average baseline F_{peak} was 37.8 +/- 5.6 kHz. At each band-limited noise frequency below the 37.5 kHz stimulus the baseline F_{peak} was well above and did not overlap with the bandwidth of the stimulus, and yet all of the stimuli caused a significant elevation in F_{peak}. The F_{peak} significantly increased from baseline to 39.1 +/- 5.2 kHz in the presence of the 17.5 kHz band-limited noise, which did not overlap in frequency with any portion of the baseline pulse. The F_{peak} at the 22.5, 27.5, and 32.5 kHz band-limited noise were also significantly elevated but did not significantly differ from the response to the 17.5 kHz band-limited noise. At the 37.5 kHz band-limited noise (the bandwidth containing the baseline F_{peak} frequency), F_{peak} increased to its maximum level of 39.9 +/- 5.2 kHz; at this stimulus frequency

roughly half the F_{peak} values would have been raised above the band-limited bandwidth. For band-limited noise frequencies that were greater than 37.5 kHz, the F_{peak} dropped to a mean value not significantly different from baseline, at which point essentially all F_{peak} values would have fallen below the bandwidth of the band-limited noise.

As mentioned previously the distribution of F_{peak} is bimodal (Fig. 2.4,b) with a peak falling on average between 30 and 35 kHz, and an second peak at 45 kHz. Very few pulses exhibited an F_{peak} near 40 kHz, accounting for the deep trough separating the two peaks. In general the effect of band-limited noise was similar to that of broadband noise, which was to cause a slight increase in the relative number of CF-FM type pulses emitted. For both the 32.5 and 47.5 band-limited noise the number of pulses with an F_{peak} between 30 and 35 kHz decreased with corresponding increases in the number of pulses with an F_{peak} at 45 kHz. Unlike the observed effect of white noise, the upper bound of the lower peak did not increase in the presence of noise. The 32.5 kHz band-limited noise caused an increase in the upper bound of the upper peak, suggesting that not only did the bats increase the number of CF-FM type pulses being emitted, but that those pulses often had a slightly higher peak frequency than normal. The increase in mean F_{peak} was therefore, as in white noise, primarily due to an increase in the number of pulses utilizing an F_{peak} that fell within the 45 kHz peak, and only slightly accounted for by an increase in the mean frequency of the upper peak. The greater change in F_{peak} observed in the 32.5 kHz band-limited can be explained by the increase in the mean of the upper peak that is not present in the 47.5 kHz band-limited.

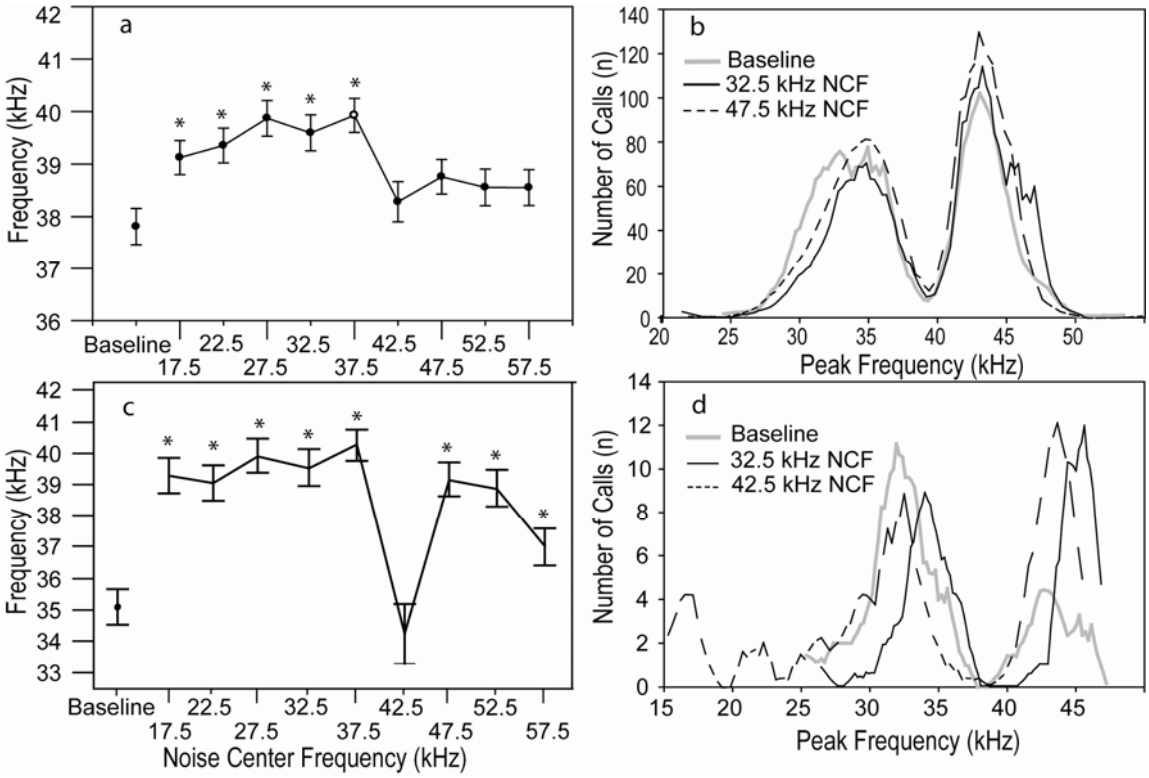

Fig 2.4. Effect of the frequency of band-limited noise. On the mean F_{peak} of 10 bats (**a,b**) and a single individual's responses (**c,d**). Asterisks denote significant difference from baseline. **a) Top Left**, the effect of band-limited noise on the mean F_{peak} is shown. The open circle represents the highest band-limited noise that overlapped with the mean initial F_{peak}. **b) Top Right, the** mean distribution for all ten individuals initially (baseline, grey line), and in the presence of a 32.5 kHz band-limited noise (solid black line) and a 47.5 kHz band-limited noise (dashed black line). **c) Bottom Left,** the effect of band-limited noise on the F_{peak} of a single individual is shown; each point represents the mean of 100 pulses. **d) Bottom Right,** the distribution of the same bat's echolocation F_{peak} initially (baseline, grey line), and in the presence of 32.5 kHz band-limited noise (solid black line) and 42.5 band-limited noise (dashed black line).

Examination of the mean response across bats revealed that a general pattern of response existed, yet upon closer inspection I observed that there was a large amount of variability within that general pattern between bats, especially in the way they regulated F_{peak} and pulse duration.

Figure 2.4c shows the individual response of one bat to the nine different band-limited noise whose behavior was not completely represented by the pooled data. Stimulus bandwidths that either included or were below the bats baseline F_{peak} resulted in an increase in F_{peak} up to a maximum frequency of 40.256 +/- 5.092 kHz at the 27.5 kHz band-limited noise. A drastic decrease in F_{peak} from its maximum value was observed at the 42.5 kHz band-limited noise. This band-limited noise represents the first interference bandwidth that is higher in frequency than the bats baseline $F_{peak,}$ 35.106 +/- 5.587 kHz. The mean F_{peak} significantly increased at the 47.5 kHz band-limited (but did not overlap with the stimulus bandwidth), and then declined again back towards baseline levels. Examination of the distribution of pulse F_{peak} for this bat showed that the underlying cause of the change in mean F_{peak} was caused by a combination of two factors. Figure 2.4c shows that band-limited noise center at 32.5 kHz caused an increase in the number of pulses in the upper mode as expected, but unlike the results from the pooled data, there was also an upward shift of the peak in both the lower and upper modes, meaning that the increase in mean F_{peak} was due to both a change in mode peak size and an increase in the mean of each mode. The 42.5 kHz band-limited noise also caused an increase in the number of pulses utilizing the upper mode, but at this stimulus frequency the peak of both the upper and lower mode was the same as baseline.

Additionally, there was in increase in the number of pulses with an F_{peak} less than 25 kHz. Thus, even though the mean F_{peak} of bats flying in 42.5 kHz interference was not significantly different from baseline, the noise still had an effect on the distribution of pulse F_{peak}. I did not observe these specific shifts in frequency in enough bats to conclude that this is a standard response for this species, however that fact that I saw it in some bats indicates that this mechanism of vocal control is possible in this species, and may be more important under other different or more natural conditions.

Another example of how the bats differed in their individual responses is shown in Figure 2.5, which shows the mean pulse durations of 3 separate individuals across all noise center frequencies. In each case, duration increased in response to an intermediate range of different band-limited noise.. However the extent of duration increase was different for each individual and appeared to vary with baseline durations. Bat 29 (Fig. 2.5,a), which had an initial mean pulse duration of 6.041 +/- 0.784 msec, increased maximally at the 32.5 kHz band-limited noise by 0.916 kHz. Bat 33 (Fig. 2.5,b) used pulses that were notably longer than average (7.413 +/- 1.046 ms) and bat34 (Fig. 2.5,c) used pulses that were shorter than average (5.211 +/- 0.839 ms). Both bats 33 and 34 increased their mean pulse duration, but the bat that started out using the longer pulses only increased duration by 0.356 ms, where as the bat using the shortest pulses (bat 34) increased its pulse durations by 1.979 ms. This may indicate that bats with shorter initial pulse durations displayed a greater magnitude of change than those with longer initial pulse durations. There was a non-significant (P=0.0830) correlation of -0.6070 between the initial pulse duration and the maximum change in duration for 9 bats.

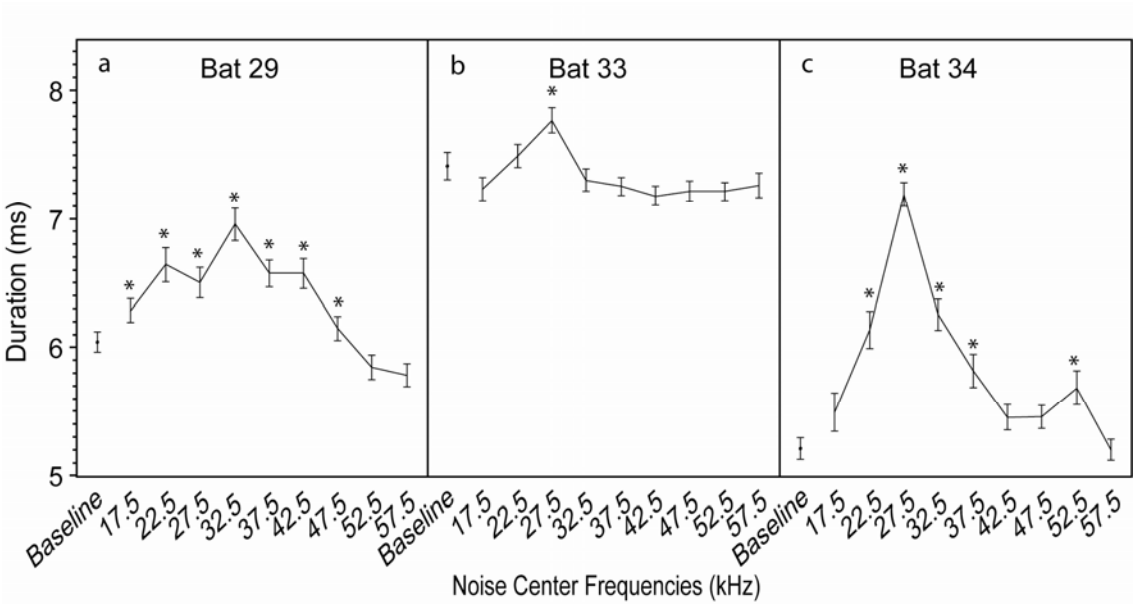

Fig. 2.5. Inter-individual variations on the effect of band-limited noise stimuli on duration. Each point signifies the mean duration of 100 pulses, asterisks denote significant difference from baseline (α=0.05). **a)** Bat 29 represents the response of an individual whose initial pulse duration was close the population mean. Bats 33 **(b)** and 34 **(c)** represent the response of individuals whose initial pulse duration was longer or shorter than the mean respectively. Note that the shorter the initial pulse duration, the greater the maximum response to band-limited noise.

The magnitude of change for the 10th individual was abnormally large and was excluded by jackknife outlier analysis. The inclusion of more individuals would likely result in a significant correlation, as the variation in duration between individuals was high.

Discussion

Previous studies have shown that many species of bats alter their echolocation pulses in response to noise (Habersetzer, 1981; Surlykke and Moss, 2000; Ibanez et al., 2004; Ratcliffe et al., 2004; Ulanovsky et al., 2004; Gillam and McCracken, 2007; Gillam et al., 2007; Bates et al., 2008). In these cases, the bats' vocal response to noise was categorized as a specialized adaptation for echolocation, however many other vertebrates that do not possess an active sensory system also alter their vocalizations in the presence of noise. It is therefore possible that some of the ways bats respond to noise are reflective of a more generalized vertebrate response to noise. I sought to determine if the JAR observed in *Tadarida brasiliensis* was indeed a separate response from the general vertebrate response to noise. In order to do so, I detailed the response of several parameters to both band-limited and broadband noise. In broadband noise, echolocation pulse amplitude, duration and bandwidth increased, and the nature and magnitude of these changes were similar to what has been reported for a variety of other vertebrates. The bats responses to band-limited noise however were collectively different from the responses to broadband noise in important ways. Duration increased similar to the response to broadband noise, but otherwise pulse parameters changed

differently; F_{peak} increased, bandwidth decreased, and amplitude remained unchanged from initial levels. The changes of F_{peak} and bandwidth in band-limited noise appear to be specific to echolocation behavior.

I compared the response of our bats in the lab using short broadband FM-pulses to reports of similar studies conducted on free-tailed bats in the field where they used long narrow-bandwidth search pulses in order to determine if the response to noise was dependent on the characteristics of the pulse being emitted. I found that the frequencies of noise that best evoked a vocal change in the laboratory differed from that previously observed in the field. The F_{peak} increased in response to band-limited noise frequencies that overlapped with the initial F_{peak} emitted in the laboratory, which is 10-20 kHz higher than the F_{peak} of search pulses emitted in the field.

In the presence of broadband noise, pulse amplitude, duration and bandwidth increased significantly from initial levels. The change in pulse bandwidth was due to both an increase in F_{start} and a decrease in F_{end}. For all of these parameters the magnitude of change was dependent on the intensity of broadband noise presented. Broadband noise did not have a significant effect on the F_{peak} or slope of the pulse.

An increase in call amplitude in response to noise is seen in many other taxa. It appears analogous to the human Lombard response, in which humans increase the loudness of their voice in response to increases in background noise (Lombard, 1911). In frogs (Penna et al., 2005), birds (Brumm and Todt, 2002; Leonard and Horn, 2005), whales (Foote et al., 2004; Scheifele et al., 2005), primates (Brumm et al., 2004; Egnor and Hauser, 2006; Sinnot et al., 1975), and bats (Simmons et al., 1978) the increase in

amplitude is accompanied with an increase in call duration. The increase in amplitude in response to noise is possibly an adaptation to increase the signal-to-noise ratio when calling in noisy environments. For echolocating bats this would increase the maximum distance an object could be detected or distinguished, as returning echoes must be louder than the background noise to be interpreted by the bat. Less consistent is the effect of broadband noise on the frequency of vocalization across taxa. Swallow begging calls in the presence of noise displayed a change in bandwidth, duration, and amplitude similar to that observed for free-tailed bat's echolocation pulses (Leonard and Horn, 2005). The fundamental frequency of human speech was significantly increased by the presence of white noise (Loren et al., 1986). In contrast, despite an increase in both call duration and amplitude, white noise had no significant effect on the fundamental frequency of cotton-top tamarins combination long calls (Egnor and Hauser, 2006). The effect of noise on call amplitude and duration appears to be highly conserved across taxa, with more variation seen in the control of spectral call parameters. The free-tailed bat's vocal response to broadband noise seems to correspond well with the general vertebrate response to noise, and I interpret the changes in duration and bandwidth to be by-products of an increase in amplitude.

Echolocation pulse duration but not loudness increased significantly in the presence of band-limited noise. The 22.5, 27.5 and 32.5 kHz band-limited noise produced a statistically significant increase that did not significantly differ from the other stimuli that overlapped with the initial echolocation pulse bandwidth. Band-limited noise frequencies that did not include echolocation pulse frequencies did not

cause a significant change in pulse duration. A frequency dependent response to noise is not unique to echolocating bats. Noise frequencies that masked the "clear calls" of the old world monkey, *Macaca*, caused a significantly greater increase in pulse duration than other frequencies tested (Sinnot et al., 1975). Mean call duration did not increase to more than 8 ms in any of the bats tested; probably because, for bats, pulse-echo delay times are also highly salient cues for regulating pulse duration and in the lab echo delay times are always short. As pulses get longer outgoing pulses may overlap in time with quickly returning echoes and thus interfere with echo perception. The magnitude of duration increases also appeared to be dependent on an individual's relative pulse duration. Individuals exhibiting longer baseline pulses tended to lengthen their pulses less in response to band-limited noise than those with shorter initial durations. Statistical analysis of this phenomenon was ambiguous due to the small number of individuals displaying either very short or very long pulses, but the trend was apparent even with the few individuals examined here.

In the field, free-tailed bats use a high proportion of long (12-16 ms) pulses for their navigation and foraging tasks (Schwartz et al., 2007), but in the lab bats used pulses of roughly 4 to 6 ms. In the field, free-tailed bats responded to the sounds of chorusing insects by increasing all frequency parameters including bandwidth and decreasing duration (Gillam and McCracken, 2007). More generally an inverse relationship between pulse duration and frequency was observed in the field (Gillam and McCracken, 2007), suggesting that these two parameters are tightly coupled. Those authors concluded that free-tailed bats do not directly adjust pulse duration, but changes in

duration occurred indirectly as a result of frequency adjustments. In the current study I observed that band-limited noises caused increases in F_{peak} and F_{end} similar in magnitude and spectral sensitivity to the field results, and likewise I observed no consistent changes in amplitude. However unlike the field measurements I observed a decrease in F_{start} (F_{max} in Gillam and McCracken, 2007) an increase in duration, and a decrease in bandwidth. These differences seem to be related to differences in the types of pulses being used, but they show that key frequency parameters (F_{peak} and F_{end}) can be elevated even while pulse durations are increasing. These results also show that starting and ending frequencies can be manipulated independently and do not always increase and decrease in unison. It seems likely that the bats' responses to band-limited noises are more complex than what would be predicted by the graded response to broadband noise. How echolocation pulse duration, frequency and bandwidth is altered in response to noise depends on what type of echolocation pulse is being used and other current acoustic conditions such as the array of pulse-echo delays that comprise the bats' acoustic scene. Generally, if the frequency of noise and the frequency of any portion of a bats echolocation pulse coincide, a change in pulse duration would be expected. The magnitude of the response will be dependent on the intensity and frequency-content of the noise. Interestingly, whether pulse duration will increase or decrease is dependent on pulse type, or at least what the duration of the pulse would have been if emitted in silence.

Unlike the response to broadband noise, bandwidth decreased in the presence of band-limited noise. The change in bandwidth was due to a combination of F_{start}

decreasing and F_{end} increasing. The decrease in bandwidth, coupled with the increase in duration, lead to a decrease in the slope of the pulse. Frequency of the band-limited noise significantly affected the magnitude of bandwidth and slope decrease. The band-limited noise frequencies that masked the largest portion of the initial echolocation pulse elicited the greatest response. It has been suggested that bats may use a type of matched filter, in which signals that "match" the expected are passed on while others are suppressed, as part of echo processing (Masters and Raver, 1996; Simmons et al., 1990). The decrease in pulse slope may allow the bat to focus on a narrower frequency range for the expected echo, improving the efficiency of its match filter to extract the pertinent echo from the masking noise.

Additionally, band-limited noise caused a significant increase in the F_{peak} for some but not all stimulus frequencies. Unlike other pulse parameters, the distribution of F_{peak} was bimodal. Changes in the mean F_{peak} were the result of a small upward shift in the mean of both modes, and an increase in the number of pulses whose F_{peak} fell in the upper mode. None of the stimuli tested caused the F_{peak} to decrease below initial levels. The stimulus frequencies that cause the greatest change in F_{peak} were typically in the range of 30 to 35 kHz, which is different from the best stimulus frequencies reported for evoking JAR in the field (Ulanovsky et al., 2004; Gillam et al., 2007) but consistent with the elevated range of mean F_{peak} values for bats echolocating in the lab. The F_{peak} of the pulses used in the lab, 35.7 +/- 5.9 kHz, was significantly higher the mean F_{peak} of search pulses recorded in the field, 26.4 +/- 1.6 kHz (Schwartz et al., 2007). The best frequency for eliciting changes in F_{peak} in the lab does not appear to correspond well with the

region of greatest sensitivity in the auditory system (Pollak et al., 1978), which instead appears to be more closely related to peak frequencies of pulses used in open spaces. Nor could similar changes in F_{peak} be evoked by varying the intensity of broadband noise. The response of F_{peak} to band-limited noise is consistent with what has been described as a jamming avoidance response (JAR) in the field for *Tadarida brasiliensis* (Gillam et al., 2007; Ratcliffe et al., 2004). The magnitude of the change in Fpeak was not closely correlated with how closely the stimulus frequency matched the initial pulse F_{peak}, but similar to the report for big brown bats (Bates et al., 2008) the upper range of stimulus frequencies at which the bats stopped elevating their F_{peak} did seem to correlate well with the stimulus passing above the baseline F_{peak} values. Presumably, the change in F_{peak} allows the free-tailed bat to maximize the signal-to-noise ratio for an important pulse component without increasing pulse amplitude. Collectively these data suggest that the best frequency for eliciting the JAR in free-tailed bats appears to change dependent on the shape of the pulse being used. Thus, the frequency component of the vocal response to noise of *Tadarida brasiliensis* is context dependent, with both the type of pulse being emitted and the nature of the noise present affecting the response.

An important question not directly addressed in these experiments was whether the bats made adjustments in their pulse acoustics due to distortions in their perception of the outgoing pulse or the returning echo. The acoustic structure of the echo will differ from the pulse because of greater atmospheric attenuation at higher frequencies and also because of doppler effects. The effects of greater attenuation at higher (start) frequencies of the echo but not the pulse might have caused bats to be more sensitive to

noises at higher frequencies since those noises would have had a greater impact on the signal to noise ratio at that bandwidth, but such a hypothesis was not supported by the data. Similarly, doppler effects, though small, shift the echo frequencies 350 to 650 Hz higher than the frequencies of the outgoing pulse. If the bats were trying to minimize overlap between the band-limited noise stimuli and the F_{Peak} of the echo, then the most effective stimulus bandwidth would have been 350 to 650 Hz higher than the recorded pulse F_{Peak}. Since I used stimulus bandwidths of 5 kHz, it was not possible from these results to discriminate such a difference. Thus, while it is possible that the bats' behavior was focused on improved echo resolution, I cannot say specifically whether the bats were cueing to the relationship between stimulus bandwidth and either pulse or echo frequencies.

Mammalian vocalizations are produced by brainstem pattern generators (Jürgens, 2002b; Jurgens and Hage, 2007). Humans alter syllable acoustics via direct projections from speech motor cortex onto respiratory and laryngeal spinal motor neurons, but the functional significance of similar pathways is unknown in other mammals (Jurgens, 2009). The change in F_{peak} exhibited by free-tailed bats appears to be a fine-tuned context dependent vocal response that might be better explained by forebrain mechanism rather than midbrain circuitry (Smotherman, 2007). Importantly, changes in F_{peak} would appear to be inherently respiratory rather than laryngeal in mechanism. By definition, F_{peak} is the frequency of the pulse at which the maximum energy is reached. Examination of the mean pulse envelope shows that the increase in F_{peak} seen in free-tailed bats occurred due to maximum energy being applied to the pulse earlier in the time

course (Fig. 2.6). This change can only be accomplished by changing the time course of expiratory force during pulse emission. Thus, changes in F_{peak} describe a respiratory modification rather than a laryngeal modification of the vocal motor pattern. Normal respiratory rhythm is controlled by brainstem regions which can be temporarily subverted by forebrain mechanisms in order to exert volitional control (Corne and Bshouty, 2005; Schulz et al., 2005). Fine volitional control of respiration has been shown to be critical in the evolution of human speech (MacLarnon and Hewitt, 1999). The free-tailed bat's F_{peak} response to band-limited noise is an insightful example of an animal utilizing a respiratory mechanism to alter the spectral components of its vocalizations, and provides an opportunity to study a mechanism for context dependent vocal control.

A comparative analysis of response to noise in bats, with particular emphasis on the predominant pulse type used, could provide insight into the evolution of vocal control in mammals. Traditionally, frequency modulated (FM) pulses are thought to be less susceptible to masking and jamming than constant frequency (CF) pulses because with such comparatively broad bandwidths there is a greater chance that enough of the pulse will be free from interference to allow for accurate echolocation (Schmidt and Joermann, 1986). Conversely, adding a concentrated burst of energy in a narrow bandwidth such as the CF component of a CF-FM pulse may improve echo discrimination in noisy environments (Schwartz et al., 2007). The tendency of our bats to switch from FM to CF-FM type pulses in noise would seem to support this hypothesis.

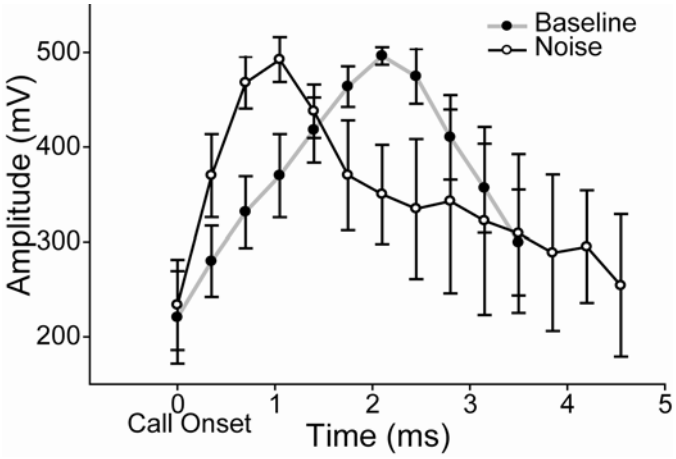

Fig 2.6. The mean pulse envelope of an individual flying in the presence and absence of noise. Pulse envelopes show the change in pulse energy, measured in mV, over the time course of the pulse, measured in ms from pulse onset. Each point represents the mean for 10 pulses initially (baseline, closed circles) and in presence of band-limited noise at the 32.5 kHz band-limited noise for one individual. The point when the maximum energy is reached defines the F_{peak} of the pulse.

Most of the current evidence of a vocal response to noise, however, is from broadband pulses. The big brown bat (*Eptesicus fuscus*) alters the F_{end} of its pulse in response to pure tones in a similar fashion to the change in F_{peak} reported here (Bates et al., 2008). In recordings of groups foraging in the field the broadband pulses of *Tadarida teniotis* changed in response to interference from conspecifics, while the much narrower band pulses of *Taphozous perforatus* were unaffected by conspecific interference (Ulanovsky et al., 2004). *Pteronotus parnellii* and *Rhinolophus ferrumequinum* both utilize a prominent CF component, and have been shown to control changes in pulse amplitude, frequency, and duration independent of each other (Kobler et al., 1985; Gaioni et al., 1990; Tian and Schnitzler, 1997). The only evidence reported of a response to noise, however, was an increase in amplitude by *Rhinolophus* of both the CF and FM component (Konstantinov et al., 1973). A more extensive survey of the response to noise across bat species must be performed in order to draw further conclusions about the evolution of vocal control.

In conclusion, broadband and band-limited noise had different effects on the pulses of *Tadarida brasiliensis*. The response to broadband noise was generally consistent with vocal adaptations for calling in noise exhibited by many vertebrates, i.e. a Lombard response, and in this case increases in amplitude and duration, and possibly frequency are likely to be biomechanically linked. Alternatively, some components of the response to band-limited noises were in many instances the opposite of their counterpart responses to broadband noise. The frequency of band-limited noise that best evoked a JAR response in the laboratory was different from values determined in field

experiments. I conclude that the jamming avoidance response displayed by the free-tailed bat is maintained in the lab and dependent on the spectral characteristics of the emitted pulse, especially the F_{peak}. In this way free-tailed bats appear to differ from big brown bats, since the JAR in those bats was most sensitive to ending frequency in echolocation pulses both in the field and lab (Bates et al., 2008). These results show that the complex vocal responses of *Tadarida brasiliensis* are context dependent, with both frequency range of stimulus and the acoustic characteristics of emitted pulses affecting the nature and magnitude of response.

CHAPTER III

EFFECT OF REDUCED DOPAMINE LEVELS ON VOCALIZATION AND THE LOMBARD RESPONSE

Introduction

Chapter II established a rapid and reliable behavioral assay of vocal plasticity in a mammal. It is hypothesized that this plasticity is dependent on extrapyramidal pathways through the basal ganglia, which are in turn dependent upon dopamine signaling in the brain. This hypothesis can be directly tested by chronically reducing dopamine levels in the brain and retesting the bats vocal plasticity using the behavioral assay described in chapter II. The drug 1-Methyl-4-phenylpyridine (MPTP) can be used to reduce the level of dopamine signaling in the basal ganglia. Since its discovery in 1983, MPTP has become an extremely useful tool for studying the biological basis of Parkinson's disease. MPTP induces a Parkinsonian-like state in humans (Davis et al., 1979; Snow et al., 2000), primates (Jenner et al., 1984), and mice (Arai et al., 1990; Sedelis et al., 2001) through metabolic neurotoxicity of dopaminergic cells in the substantia nigra pars compacta (Bradbury et al., 1986). This gives rise to Parkinson's motor disorders that are indistinguishable from natural onset of the disease (for review see (Dauer and Przedborski, 2003). By using this approach in bats I hope to identity important functions of the basal ganglia in the control of vocalization.

Hypokinetic dysarthria is a suite of symptoms that is often associated with the early stages of Parkinson's disease. Characterized by harsh and breathy voice, a reduced

voice bandwidth, reduced articulation, and reduced voice volume (hypophonia) (Sapir et al., 2008). One major hindrance in the development of treatments for these speech-related symptoms is the lack of a parkinsonian animal model that displays similar vocal symptoms. As the BG is thought to be mainly involved in control of complex vocal behaviors, such as vocal learning, speech and singing (Jürgens, 2009), animals with limited vocal repertoires such as rodents and primates haven't contributed much to the study hypokinetic dysarthria. The Mexican free-tailed bat (*Tadarida brasiliensis*) displays a diverse repertoire of volitional communication vocalizations (Bohn et al., 2008; Bohn et al., 2009), but for the purpose of this study it is the plasticity of their echolocation behavior that is particularly interesting. Isolated free-tailed bats call continuously at high rates and have now been shown to make rapid changes in call acoustics in response to sensory feedback, making it a prime candidate for the study of parkinsonian vocal disorders.

Mexican free-tailed bats respond to acoustic interference in a complex and context dependent manner. When presented with a narrow-band stimulus they are capable of altering the frequency characteristics of there echolocation calls to maximize the signal-to-noise-ration of the returning echo without increasing call amplitude, a jamming avoidance response (JAR) (Tressler and Smotherman, 2009). When presented with a broadband noise stimulus, the bats display a Lombard response. As seen in many other mammals including humans, the Lombard response is characterized by an increase in call amplitude in response to background noise (Lombard, 1911). The magnitude of the response is linearly proportional to the loudness of the interfering noise. I will focus

on the Lombard vocal response in this experiment because of its relative simplicity, the consistency and reliability of this vocal response across animals, the fact that this behavior is shared by bats, cetaceans, primates and humans, and because of clinical evidence that PD affects the Lombard response in humans.

Based on the known function of the BG and the observation of parkinsonian like conditions in other organisms, it is hypothesized that administration of MPTP will result in spontaneous echolocation calls displaying decreased amplitude and reduced bandwidth. Additionally, as the BG is known to play a role in sensory-motor integration, it is predicted that MPTP will alter the bats vocal response to noise. Since no previous studies have measured the effects of MPTP on bat behavior or physiology, this experiment requires an initial series of tests to determine the appropriate dosage and timing of experiments.

Methods

Animal husbandry

Three Mexican free-tailed bats, *Tadarida brasiliensis mexicana*, were caught wild from a year round roost on the campus of Texas A&M University and housed in the Texas A&M Department of Biology vivarium facility. Bats were kept on a phase-shifted 12/12 day/night cycle, with vivarium lights turning off at 12:00pm. The bat vivarium was a temperature and humidity controlled room that was large enough to allow the bats to fly freely. Bats were trained to feed themselves and had to fly daily to obtain food. The bats were fed a diet of mealworms supplemented with vitamins, minerals and essential fatty acids.

Acoustic stimuli

Acoustic stimuli consisting of broadband noise was generated digitally with Tucker-Davis Technology (TDT) system III hardware and the openEX software v5.4. The broadband noise was digitally filtered to present a total signal bandwidth spanning a range of 15 to 100 kHz, which covered the entire range of the two loudest harmonic components of *Tadarida brasiliensis'* echolocation pulses. Stimuli were played through a Sony amplifier (model # STR-DE598) driving a 4-speaker array composed of 2 Pioneer Ribbon Tweeter (ART-55D/301080) and 2 Pioneer Rifle Tweeter (ART-59F/301081) Speakers were arranged so that 1 Ribbon and 1 Rifle tweeter always projected directly at the subject, either stationary or in flight. Each speaker provided a flat (±3 dB) output at 85 dB SPL across the principal frequency range of interest, roughly 15 to 60 kHz. The bats' echolocation pulses ranged in intensity from 80 to 115 dB SPL at rest.

Acoustic recording set-up

All experiments were performed in an 8 meter long by 2 meter wide by 3 meter high flight tunnel lined with sound-absorbing 4-inch acoustic foam (Sonex ©,model UNX-4), with the lights off. Recordings were made using a Bruel & Kjaer Free-field ¼" microphone (Type 4939).

For experiments on stationary individuals, bats were placed in a 14x14x5cm wire mesh cage. The microphone was positioned 15cm from the bottom of the cage 9.75cm in from the cage corner. The placement of the microphone combined with the cage dimensions ensured that the experimental subject was facing the microphone from a

12cm distance for the experiment. This method was used rather than head restraint to obtain the most natural echolocation behavior possible while still ensuring accurate measures of pulse intensity. Recorded intensity of acoustic stimuli was minimized by placing sound absorptive foam around the microphone on all sides except that facing the bat, which facilitated the digital extraction of echolocation pulses from the background noise.

For experiments on flying individuals, bats were allowed to fly along the long axis of the recording chamber freely. The microphone was placed in the center of the chamber 60cm above the floor. The placement of the microphone was coordinated with the positions and directionality of the speakers to minimize the recorded intensity of the stimuli while maximizing the recorded intensity of the bat pulses, which facilitated the digital extraction of echolocation pulses from the background noise.

Incoming signals were digitized with a National Instruments DAQmx, NI PCI-6251 (200 kHz, 16 bit sample rate), and viewed with Avisoft Recorder v3.0. Pulse duration and intensity were analyzed using SASLab Pro v4.39 using the methods of Tressler and Smotherman (2009). Additionally, the rate at which pulses were emitted, expressed as calls-per-minute (calls/min) were recorded.

Pharmacology

Solid 1-Methyl-4-phenylpyridine powder was obtained from Sigma-Aldrich, St. Louis, MO (catalog # M0896), and dissolved in physiological saline. A 0.1ml intraperitoneal injection resulted in a final dose of 5mg/kg. Fresh solution was made for each injection. Saline injections were used for controls.

Experimental procedure

Effect of MPTP over time

In order to determine the time course of MPTP effect and to test the effect of multiple injections over time, 4 MPTP injections were administered to each subject, separated by 8 days of observation. Each round of the experiment was composed of an MPTP injection on day 1, and experimental recordings taken one hour, one day, and 7 days after injection. The 4 rounds were compared to determine if there was a cumulative effect.

Motor control assays

In order to determine if MPTP injections were having an effect on gross motor control, standard motor control assays were performed at each experimental session (Muralikrishnan and Mohanakumar, 1998; Sikiric et al., 1999). Specifically, subjects were placed individually into a 14x14x5cm wire mesh arena, and the time it took the subject to move all 4 limbs and climb onto the wall of the arena were recorded and scored on a 0-4 scale as follows. Completing the task in less than 1s received a score of 0, 1s-1m received a score of 1, 1m-2m received a score of 2, 2m-3m, received a score of 3, greater than 3m received a score of 4. Untreated individuals will perform these tasks in less than a second. Additionally, the presence and degree of tremor was scored by trained observers on a 0-4 scale. Finally, any other unanticipated changes in motor behavior observed but not reflected by these measurements (for example unnatural postures or evidence of sensory deficits) were documented by observers who were experienced with these bats' normal behavior.

Effect of MPTP on vocalization and Lombard response in stationary bats

Three individual Mexican free-tailed bats, *Tadarida brasiliensis*, were selected at random for the captive colony. One hour after a 0.1ml intraperitoneal injection of physiological saline (day 0), individuals were recorded in the presence and absence of broadband noise, serving as a baseline control. After at least 24 hours rest, the same individuals were then administered an MPTP injection and its vocal behavior was retested following the above time course. Recordings and motor control assessments were taken 1 hour, 1 day, and 7 days after MPTP injection (day 1, day 2, and day 7 respectively).

Each experimental recording session lasted one hour, or until over 1000 echolocation, pulses had been recorded. Recording sessions were divided equally into periods of silence and periods of broadband noise presented to the bats in a balanced pseudo-random fashion.

The effect of broadband noise on the subject's echolocation pulse parameters (Lombard response) was determined by subtracting the mean value of each acoustic parameter in the presence of noise from the mean value in the absence of broadband noise for each parameter for each subject. The effect of MPTP on the Lombard response was determined by subtracting the mean Lombard response of individuals treated with MPTP from same individuals treated with saline for each parameter for each subject.

Effect of MPTP on vocalization and Lombard response in flight

The same procedure as for stationary recordings was used Except that individuals were recorded while flying 1 hour after saline injection and 1 hour after MPTP injection

only. Previous work in mice has shown that the effects seen immediately after injection disappear within the first hour (Sedelis et al., 2001). By delaying the start of recordings for all MPTP experiments, I hope to ensure that alterations in motor patterns observed are the result of chronic changes in nigrostriatal DA levels, and more closely resemble the gradual long term DA loss in Parkinson's disease.

Statistical analysis

All statistical procedures were performed utilizing SAS-JMP v7.0.7 at the α=0.05 level. Overall effects of MPTP on echolocation pulse parameter in silence and on Lombard response were analyzed by MANOVA. If significant, individual parameters were compared with ANOVA, and difference between individual treatments within a parameter were tested with a student t-test, α=0.05. Motor control assays were analyzed using a non-parametric chi-squared test. Results are given as means ± S.D. unless otherwise noted.

Results

Effect of MPTP on behavioral motor assays

There was no significant effect of MPTP on either the time taken to move 4 limbs (P=0.5165), time to cross the arena (P=0.4860), or on the presence of tremor

(P=0.4317). Only 1 bat showed the presence of tremor on the day of and the day after the first injection (day 1 and 2). At this dosage, no other visible signs of MPTP-induced motor deficits were observed. Preliminary studies with higher doses (unpublished) caused such severe motor deficits that treated bats were unable to vocalize, precluding the possibility of experimentation at that dose. Bats remained able to self-feed, and water, as well as climb and fly throughout the course of the experiment.

Effect of multiple MPTP injections over time

In order to test for an effect of multiple MPTP injections over time, the effects of MPTP on echolocation pulse variables were compared across injection events (i.e. rounds). The effect of MPTP on echolocation pulse characteristics did not significantly change with subsequent injections after the first (MANOVA P=0.1141). The measurement of each parameter and the change induced by MPTP for all rounds can be seen in tables 3.1 and 3.2 respectively. Round was removed as a factor from all subsequent statistical analysis, and only the effect of MPTP on echolocation pulse parameters over 7 days was considered.

Table 3.1. Echolocation call parameters following injection of saline (day-0) or 5mg/kg MPTP. Bats were administered MPTP on days 1, 8, 16, and 24. Observations and recordings only were perfomed on days 2, 7, 9, 15, 12, 23, 25, and 31. Each value is the mean of three bats and the standard deviation. Noise condition represents echolocation in the presence of broadband noise broadcasting at 85 dB-SPL.

Day	Treatment	Duration (mSec)	S.D.	Amplitude (dB-SPL)	S.D.	Start (kHz)	S.D.	End (kHz)	S.D.	Peak (kHz)	S.D.	Bandwidth (kHz)	S.D.	Rate (calls/min)	S.D.
0	noise	4.656	0.837	113.555	4.992	46.196	1.179	27.987	1.772	34.569	2.626	18.267	0.580	284.837	61.162
0	silence	3.534	0.482	123.227	4.356	41.166	2.118	27.973	3.940	31.684	3.700	13.533	3.518	231.689	192.778
1	noise	1.544	0.490	103.870	1.702	15.329	0.848	15.133	0.760	15.426	0.730	1.076	0.408	6.719	2.773
1	silence	1.798	0.162	104.026	2.163	17.106	3.660	16.641	2.861	16.915	3.378	1.616	1.349	4.848	2.298
2	noise	4.711	0.802	108.570	5.484	44.814	2.812	27.938	2.086	33.300	2.508	17.069	1.168	42.855	23.437
2	silence	2.412	0.453	117.672	6.228	33.467	1.995	27.051	1.320	29.491	2.163	7.975	2.505	20.548	8.933
7	noise	5.423	0.679	112.492	4.501	52.581	0.752	30.250	4.277	34.288	5.691	22.403	3.627	42.133	41.047
7	silence	3.585	0.360	124.388	3.370	44.305	0.837	29.271	2.387	31.765	4.117	15.242	3.519	34.131	34.580
8	noise	2.461	0.898	106.474	4.897	19.407	8.289	16.496	3.055	17.776	5.066	3.647	5.482	8.213	5.384
8	silence	2.030	0.042	111.182	7.008	14.921	0.440	15.147	0.249	15.119	0.482	0.712	0.357	4.634	1.299
9	noise	3.491	1.702	104.388	2.693	29.967	15.008	21.079	6.742	25.001	11.169	8.971	8.385	48.470	75.045
9	silence	2.555	0.673	107.904	5.854	26.587	12.421	22.099	7.796	24.446	10.417	5.151	5.009	55.170	77.604
15	noise	4.174	0.469	109.349	6.341	40.307	1.858	26.938	0.404	31.330	3.121	13.782	1.891	142.870	102.544
15	silence	2.904	0.283	114.770	5.719	34.248	2.902	27.524	0.564	30.141	3.194	8.446	1.304	143.706	100.956
16	noise	2.618	1.470	103.277	1.179	23.106	15.628	18.359	6.567	20.626	10.383	5.721	8.299	28.872	34.876
16	silence	2.216	0.827	105.497	3.052	21.471	12.622	18.157	6.471	19.780	9.334	3.927	5.693	22.919	31.210
17	noise	3.552	1.561	109.318	5.429	33.191	15.483	23.287	6.719	24.725	8.330	10.596	8.824	100.732	83.684
17	silence	2.567	0.405	110.676	10.217	31.901	5.337	24.575	2.125	26.717	2.698	8.324	3.381	107.029	87.127
23	noise	5.160	0.424	110.111	7.670	46.117	2.236	28.113	2.811	33.055	1.874	18.824	0.963	124.249	111.327
23	silence	3.426	1.121	121.106	4.356	38.913	5.916	27.116	1.725	29.122	1.689	11.998	5.840	204.747	48.420
24	noise	2.890	1.096	105.544	4.291	20.950	6.322	17.066	2.735	19.740	4.937	4.626	4.207	8.552	6.001
24	silence	1.960	0.110	112.435	8.749	15.216	0.287	15.382	0.530	15.302	0.424	0.821	0.447	5.258	2.006
25	noise	3.630	1.283	99.796	0.892	29.786	14.950	20.066	5.381	23.527	8.097	10.339	9.524	4.940	1.388
25	silence	2.934	0.191	110.892	8.894	22.384	9.478	20.680	3.579	21.800	5.751	5.749	2.424	14.883	18.795
31	noise	5.590	0.054	111.241	6.504	46.150	0.150	27.002	1.611	33.461	7.745	19.373	1.819	103.090	75.836
31	silence	3.614	0.413	120.930	2.885	39.800	2.053	26.050	0.257	28.494	1.172	14.136	1.788	153.153	27.715

Table 3.2. Effect of MPTP on echolocation call parameters by round by day. Each value is the mean difference for 3 bats between the saline and MPTP injections. MPTP injections occurred on day 1 of each round.

Day	Round	Treatment	Duration (mSec)	S.D.	Amplitude (dB-SPL)	S.D.	Start (kHz)	S.D.	End (kHz)	S.D.	Peak (kHz)	S.D.	Bandwidth (kHz)	S.D.	Call Rate (calls/min)	S.D.
1	1	silence	-1.973	0.078	-9.685	6.180	-24.060	5.775	-11.332	5.966	-14.769	4.728	-11.917	4.063	-278.118	63.654
1	1	noise	-3.112	0.565	-19.201	5.424	-30.867	1.333	-12.854	1.609	-19.143	3.327	-17.191	0.331	-226.841	190.976
2	1	silence	-1.358	0.394	-4.985	6.367	-7.699	0.605	-0.922	2.717	-2.192	1.689	-5.558	3.438	-241.982	80.601
2	1	noise	0.055	0.605	-5.555	4.943	-1.381	1.781	-0.049	0.451	-1.269	0.381	-1.198	1.571	-211.141	200.982
7	1	silence	-0.224	0.458	-0.766	2.521	1.916	0.835	0.609	7.698	0.036	1.115	1.018	8.197	-242.704	88.072
7	1	noise	0.287	0.557	0.307	2.424	5.966	2.064	1.562	6.102	-0.625	2.073	4.396	4.145	-197.558	227.011
1	2	silence	-1.741	0.138	-7.081	9.256	-26.245	1.681	-12.826	4.039	-16.565	3.580	-12.821	3.536	-276.624	63.112
1	2	noise	-2.194	1.734	-12.045	10.879	-26.789	9.098	-11.491	4.609	-16.794	6.009	-14.620	5.038	-227.055	191.653
2	2	silence	-1.216	0.596	-9.167	6.151	-14.579	13.024	-5.874	11.687	-7.238	6.971	-8.382	1.661	-236.367	36.120
2	2	noise	-1.164	1.818	-15.323	10.055	-16.228	15.997	-6.908	8.270	-9.569	8.636	-9.296	8.033	-176.519	264.703
7	2	silence	-0.867	0.227	-4.206	1.653	-6.918	4.752	-0.449	3.432	-1.543	0.781	-5.087	4.350	-141.967	54.808
7	2	noise	-0.482	0.379	-8.457	4.177	-5.889	2.902	-1.049	1.384	-3.240	0.696	-4.485	1.402	-87.983	98.190
1	3	silence	-1.554	0.735	-10.278	5.168	-19.695	11.668	-9.816	9.561	-11.903	6.355	-9.606	2.278	-255.965	34.139
1	3	noise	-2.038	1.323	-17.730	6.646	-23.090	16.102	-9.628	7.255	-13.943	7.910	-12.546	8.238	-208.770	222.025
2	3	silence	-1.203	0.310	-4.237	2.607	-9.265	3.790	-3.398	3.815	-4.966	2.559	-5.209	2.291	-184.106	104.877
2	3	noise	-1.103	0.806	-12.551	7.705	-13.004	14.837	-4.700	5.408	-9.845	7.476	-7.672	9.216	-124.660	271.138
7	3	silence	-0.344	1.090	-3.444	5.746	-2.253	3.880	-0.857	2.327	-2.561	2.039	-1.535	5.576	-160.588	93.890
7	3	noise	0.504	0.479	-2.121	4.433	-0.078	1.083	0.126	1.564	-1.515	2.699	0.557	0.407	-26.942	240.013
1	4	silence	-1.811	0.187	-8.011	9.282	-25.950	2.353	-12.591	4.275	-16.382	3.447	-12.712	3.790	-276.286	60.008
1	4	noise	-1.766	1.917	-10.792	12.258	-25.246	7.455	-10.921	4.499	-14.829	4.156	-13.641	3.640	-226.431	191.471
2	4	silence	-0.836	0.262	-13.759	5.592	-18.782	8.951	-7.293	6.491	-9.884	3.234	-7.783	1.591	-279.898	61.311
2	4	noise	-1.026	1.567	-12.334	13.239	-16.409	15.995	-7.921	7.115	-11.042	6.039	-7.928	9.171	-216.806	211.005
7	4	silence	-0.195	0.511	-2.017	0.518	-2.589	2.051	-2.612	5.053	-3.235	4.059	-0.088	6.466	-181.499	162.330
7	4	noise	0.453	0.176	-3.151	2.909	-0.466	1.162	-1.686	3.436	-1.452	4.127	1.366	2.337	6.619	147.836

Effect of MPTP on echolocation call parameters in stationary bats

MPTP had a significant effect on echolocation pulse parameters (MANOVA $P<0.0001$).

Saline/Baseline

After saline injection, the echolocation pulses emitted in silence had a mean duration of 3.534 ± 0.482ms, amplitude of 123.227 ± 4.356dB-SPL, F_{Start} of 41.166 ± 2.118kHz, F_{End} of 27.973 ± 3.940kHz, F_{Peak} of 31.684 ± 3.700kHz, bandwidth of 13.533 ± 3.518kHz and the bats displayed a call rate of 231.689 ± 192.778 calls-per-minute (call/min).

Duration

One hour after injection (day 1), duration of pulses in silence had decreased significantly ($P<0.0001$) by a mean of 1.770 ± 0.336ms to 2.001 ± 0.395ms. One day after injection (day 2) pulse duration was significantly higher than day 1 ($\alpha=0.05$) at 2.617 ± 0.444ms, but still an average of 1.154 ± 0.404ms less than the mean call duration obtained with saline injections. No significant change from baseline was found one week after injection (day 7), when pulse duration was only 0.447 ± 0.644ms less than saline levels at 3.339 ± 0.653ms (Fig. 3.1,a).

Amplitude

Mean echolocation pulse amplitude in silence on day 1 was 104.791 ± 3.205dB-SPL, a significant decrease ($P=0.0004$) of 8.764 ± 6.695dB-SPL. There was no significant change between day 1 and day 2 ($\alpha=0.05$), which had a mean amplitude of

105.518 ± 5.296dB-SPL. By day 7, pulse amplitude was only 2.852 ± 3.245dB-SPL less than saline levels, with a mean value of 110.585 ± 5.518dB-SPL (Fig 3.1,b).

Call rate

MPTP caused a significant decrease in the rate of echolocation pulses emitted in silence (P=0.0489). Call rate decreased by 271.748 ± 49.179 call/min on day 1, to a significantly low rate of 9.415 ± 15.667calls/min. On Day 2 call rate remained reduced at 49.408 ± 63.409calls/min, 235.588 ± 73.289calls/min less than saline. Changes in call rate on day 1 and 2 were not significantly different from each other (α=0.05). On day 7, call rate was 181.707 ± 90.962 calls/min less than saline at 132.187 ± 86.110calls/min (Fig 3.1,c).

Frequency characteristics

F_{Start}, F_{End}, F_{Peak}, and bandwidth are all highly correlated with each other. MPTP significantly decreased all 4 parameters (P<0.0001 for all frequency parameters) and the change each day was significantly different from the other days (α=0.05). F_{Start} in silence was 17.179 ± 6.239kHz on day 1, 28.585 ± 8.448kHz on day 2, and 38.769 ± 4.905kHz on day 7, a decrease of 23.987 ± 6.309kHz, 12.581 ± 8.316kHz, and 2.886 ± 4.428kHz respectively. F_{End} in silence decreased by 11.641 ± 5.563kHz to 27.972 ± 3.940 on day 1, by 4.372 ± 6.552kHz to 23.601 ± 4.579kHz on day 2, and by 0.792 ± 3.803kHz to 27.456 ± 1.608 on day 7.

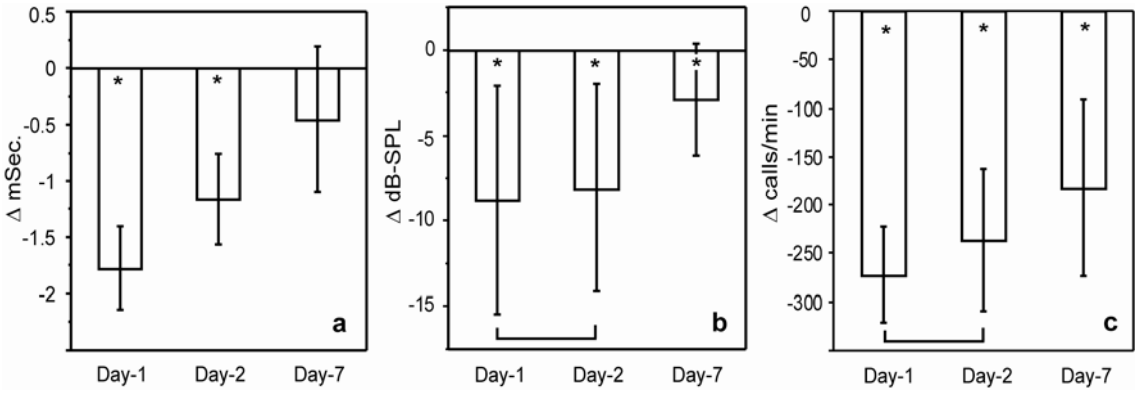

Fig 3.1. The effects of MPTP on echolocation call duration (a), amplitude (b), and call rate (c) in silence. Each bar represents the mean change from saline of 3 bats, error bars are one standard deviation from the mean. MPTP had a significant effect on duration ($P<0.0001$), amplitude ($P=0.0008$), and call rate ($P=0.0489$). Results marked with an asterisk are significantly different from zero, those connected by a solid line are NOT significantly different from one another ($\alpha=0.05$).

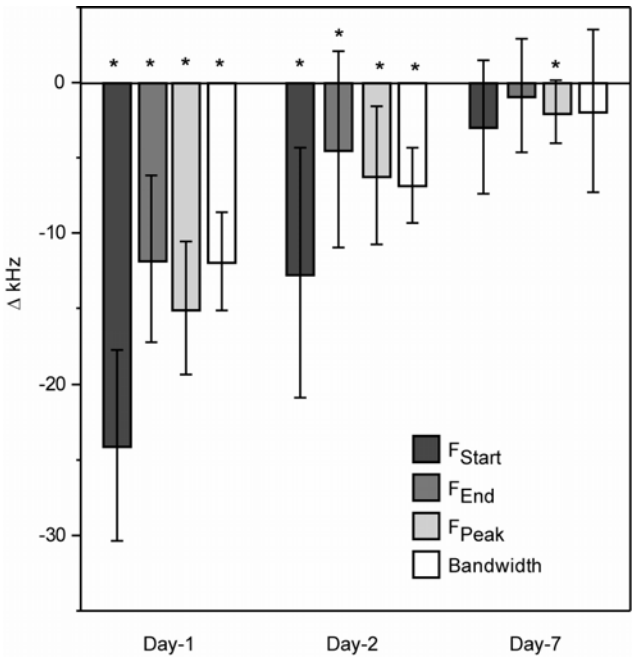

Fig 3.2. The effects of MPTP on echolocation call frequency characteristics in silence. Each bar represents the mean change from saline of 3 bats, error bars are one standard deviation from the mean. Results marked with an asterisk are significantly different from zero, those connected by a solid line are NOT significantly different from one another ($\alpha=0.05$). MPTP significantly reduced all frequency characteristics ($P<0.0001$ for all parameters).

Day 1 F_{Peak} in silence was 14.905 ± 4.439kHz less than saline levels at 16.779 ± 4.669kHz, day 2 was 6.070 ± 4.606kHs less at 25.614 ± 6.057kHz, and on day 7 F_{Peak} was 1.871 ± 2.108kHz less than baseline at 29.831 ± 2.523kHz. Echolocation pulse bandwidth in silence was 1.769 ± 2.848kHz on day 1, 6.800 ± 3.302kHz on day 2, and 12.009 ± 4.153kHz on day 7, a decrease of 11.764 ± 3.264kHz, 6.733 ± 2.473kHz, and 1.801 ± 5.410kHz respectively (Fig 3.2).

Effect of MPTP on Lombard response, stationary

MPTP had a significant effect on how bats altered their echolocation pulses in response to broadband noise (MANOVA P=0.0072).

Saline/Baseline

The echolocation pulses emitted in noise had a mean duration of 4.656 ± 0.837ms, amplitude of 113.555 ± 4.992dB-SPL, F_{Start} of 46.196 ± 1.179kHz, F_{End} of 27.987 ± 1.772kHz, F_{Peak} of 34.569 ± 2.626kHz, bandwidth of 18.267 ± 0.580kHz, and they displayed a call rate of 284.837 ± 61.162 calls/min. The resultant response to noise for saline injections was an increase in duration of 1.122 ± 0.388ms, an increase in amplitude of 9.672 ± 2.174dB-SPL, an increase in F_{Start} of 5.030 ± 1.672kHz, an increase of F_{End} of 0.014 ± 2.465kHz, an increase in F_{Peak} of 2.885 ± 1.253kHz, a bandwidth increase of 4.734 ± 3.371kHz, and a call rate increase of 53.148 ± 236.562calls/min.

Duration

The mean echolocation pulse duration in broadband noise was 2.378 ± 1.040ms on day 1, 3.846 ± 1.289ms on day2, and 5.003 ± 0.702ms on day 3 resulting in a change

in response to noise of -0.791 ± 1.028ms, 0.624 ± 1.180ms, and 0.653 ± 1.080ms on day 1, 2, and 3 respectively (Fig 3.3,a), a significant change from the baseline response (P=0.0042). Days 2 and 7 are not significantly different from each other (α=0.05).

Amplitude

MPTP significantly decreased the magnitude of amplitude change caused by broadband noise (P=0.0473). Echolocation pulse amplitude in broadband noise was 108.285 ± 6.280dB-SPL on day 1, 111.786 ± 7.795dB-SPL on day 2, and 119.826 ± 5.257db_SPL on day 7. The resultant effect on the response to broadband noise was a decrease of 5.271 ± 7.318dB-SPL on day 1, a decrease of 0.299 ± 6.592dB-SPL on day 2, and an increase of 1.638 ± 5.267dB-SPL on day 7 (Fig 3.3,b). Days 2 and 7 are not significantly different from each other (α=0.05).

Call rate

MPTP significantly changed the response of call rate to broadband noise (P=0.003) by -166.728 ± 219.232CpM on day 1 to 13.089 ± 18.041calls/min. Call rate increased on day 2 by 79.230 ± 44.842CpM resulting in a higher than saline response to noise and a call rate of 49.249 ± 60.556calls/min. On day 7 the call rate response to noise was 114.360 ± 191.514calls/min less than saline, with a mean call rate in noise of 103.085 ± 84.957calls/min (Fig 3.3,c).

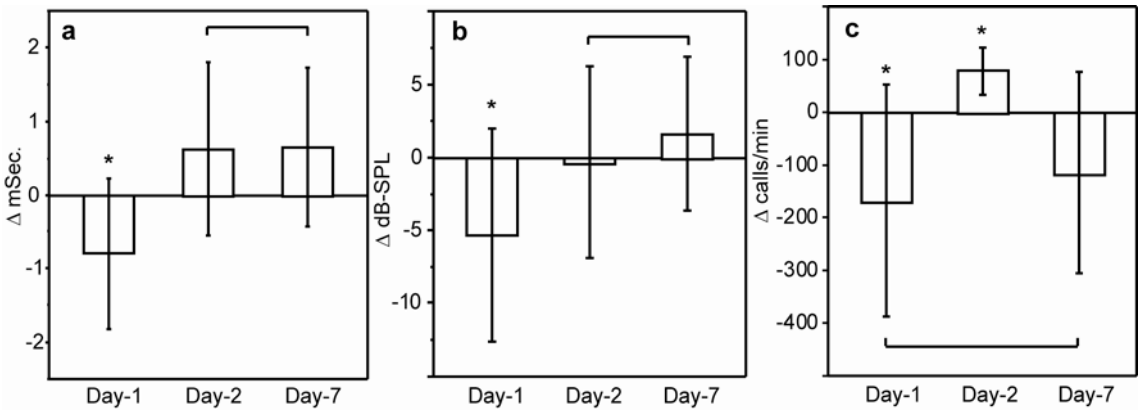

Fig 3.3. Effect of MPTP on the vocal response to noise for echolocation call duration (a), amplitude (b), and call rate (c). Each bar represents the mean change in the response to noise of three bats. Error bars are constructed with one standard deviation. MPTP had a significant effect on the response to noise for duration (P=0.0042), amplitude (P=0.0473), and call rate (P=0.0030). Results marked with an asterisk are significantly different from zero, those connected by a solid line are NOT significantly different from one another (α=0.05).

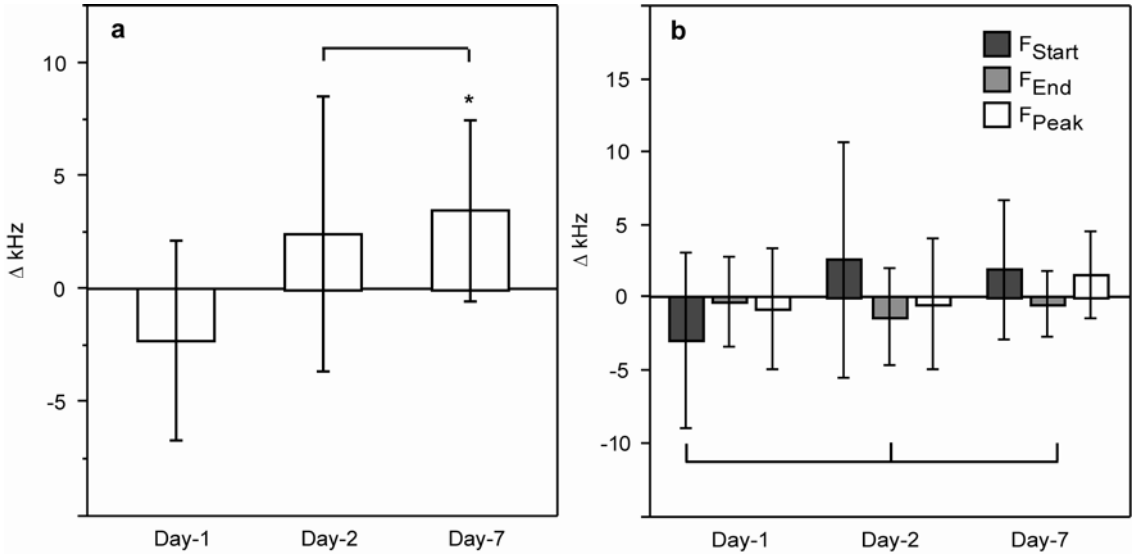

Fig 3.4. Effect of MPTP on the vocal response to noise for echolocation call bandwidth (a) F_{Start}, F_{End}, and F_{Peak} (b). Only the change in bandwidth was significantly effected by MPTP (P=0.0228). Each bar represents the mean change in the response to noise of three bats, error bars are constructed with one standard deviation from the mean. Results marked with an asterisk are significantly different from zero, those connected by a solid line are NOT significantly different from one another (α=0.05).

Frequency characteristics

Bandwidth was the only frequency characteristic response to noise which was significantly effected by MPTP (bandwidth P=0.0228, F_{Start} P=0.0999, F_{End} P=0.6749, F_{Peak} P=0.3574). Day 1 bandwidth in noise was 3.767 ± 4.945kHz, 11.743 ± 7.376kHz on day 2, and 18.137 ± 3.701kHz on day 7, resulting in a change in the response to broadband noise of -2.235 ± 4.383kHz on day 1, 2.425 ± 6.094kHz on day 2, and 3.482 ± 4.018kHz on day 7 (Fig 3.4,a). F_{Start} in broadband noise was 19.698 ± 8.551kHz on day 1, 34.440 ± 12.952kHz on day 2, and 45.673 ± 4.719kHz on day 7. The effect of MPTP on the response to noise for F_{Start} was a 2.881 ± 5.975kHz decrease on day 1, a 2.618 ± 8.100kHz increase on day 2, and a 1.946 ± 4.809kHz increase on day 7 (Fig 3.4,b). F_{End} in broadband noise was 16.764 ± 3.531kHz on day 1, 23.092 ± 5.705kHz, 27.966 ± 2.416kHz on day 7. The change in the response to noise was -0.289 ± 3.112kHz on day 1, -1.303 ± 3.349kHz on day 2, and -0.430 ± 2.287kHz on day 7 (Fig 3.4,b). The effect of MPTP on the response to noise of F_{Peak} was a 0.762 ± 4.173 decrease on day 1, a 0.422 ± 4.505kHz decrease on day 2, and an increase of 1.586 ± 3.044kHz on day 7 (Fig 3.4,b). Mean F_{Peak} in broadband noise was 18.392 ± 5.758kHz, 26.638 ± 8.052kHz, and 32.865 ± 3.812kHz for days 1, 2 and 7 respectively.

Effect of MPTP on echolocation pulse characteristics and Lombard response in flight

MPTP had no significant effect on echolocation pulse characteristics (P=0.1967) or Lombard response emitted by bats in flight.

Saline/Baseline

For echolocation pulses emitted by bats flying in silence after saline injection duration was 3.944 ± 0.722ms, amplitude was 102.937 ± 3.761dB-SPL, F_{Start} was 39.962 ± 6.410kHz, F_{End} was 26.008 ± 3.590kHz, F_{Peak} was 32.864 ± 7.452kHz, bandwidth was 13.954 ± 4.691kHz, and a call rate of 177.268 ± 29.262calls/min. For pulses emitted in noise the duration was 4.213 ± 0.509ms, amplitude was 107.567 ± 0.906dB-SPL, F_{Start} was 40.543 ± 5.464kHz, F_{End} was 25.687 ± 2.515kHz, F_{Peak} was 34.621 ± 6.973kHz, bandwidth was 14.856 ± 3.083kHz and a call rate of 352.415 ± 137.134calls/min (Fig 3.5). The response to noise was an increase of 0.270 ± 0.757ms in duration, an increase of 4.630 ± 3.743cB-SPL for amplitude, a increase of 0.581 ± 3.062kHz for F_{Start}, a decrease of 0.321 ± 1.950kHz for F_{End}, a increase of 1.757 ± 1.988kHz for F_{Peak}, an increase of 0.902 ± 4.811kHz for bandwidth, an increase in call rate of 298.011 ± 134.862calls/min.

MPTP

After MPTP treatment, echolocation pulse duration for bats flying in silence was 3.099 ± 0.546ms, call amplitude was 104.427 ± 2.675dB-SPL, F_{Start} was 39.430 ±

5.518kHz, F_{End} was 27.524 ± 6.306kHz, F_{Peak} was 33.608 ± 8.620kHz, bandwidth was 11.906 ± 2.713kHz, and a call rate of 171.628 ± 135.593calls/min. For bats flying in broadband noise duration was 4.116 ± 1.154ms, amplitude was 109.666 ± 0.653dB-SPL, F_{Start} was 42.284 ± 6.639kHz, F_{End} was 25.155 ± 3.008kHz, F_{Peak} was 37.025 ± 9.692kHz, bandwidth was 17.128 ± 3.743kHz, and call rate was 469.639 ± 10.495calls/min (Fig 3.5). The effects of MPTP on the responses to noise was a increase in the duration response of 0.747 ± 0.367ms, an increase in the amplitude response of 0.608 ± 0.751dB-SPL, an increase in the F_{Start} response of 2.273 ± 4.058kHz, a decrease in the F_{End} response of 2.047 ± 2.725kHz, an increase in the F_{Peak} of 1.661 ± 3.009kHz, an increase in the bandwidth response of 4.320 ± 3.685kHz, and a decrease in the call rate response of 122.864 ± 22.718calls/min (Fig. 3.6).

Discussion

The action of MPTP on the dopamine producing cells in the SNc in mammals is well established (Smeyne and Jackson-Lewis, 2005), and from the results of this study it

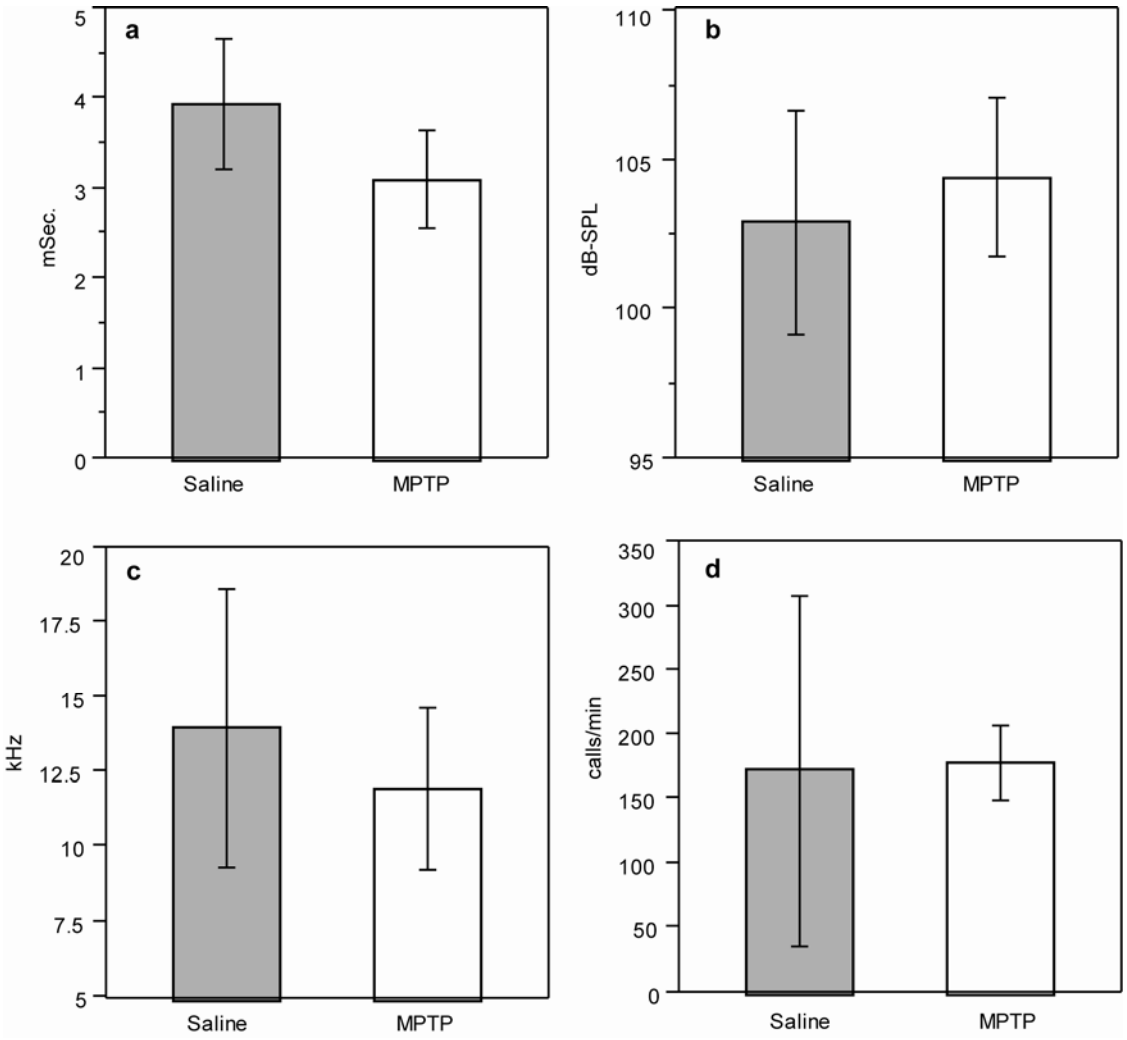

Fig 3.5. Mean echolocation call duration (a), amplitude (b), bandwidth (c), and call rate (d). Bats were flying in silence after saline (grey bars) or MPTP (white bars) injection. Each bar represents the mean of 3 bats, error bars are constructed with 1 standard deviation from the mean. There was no significant effect on any call parameter (P=0.1967).

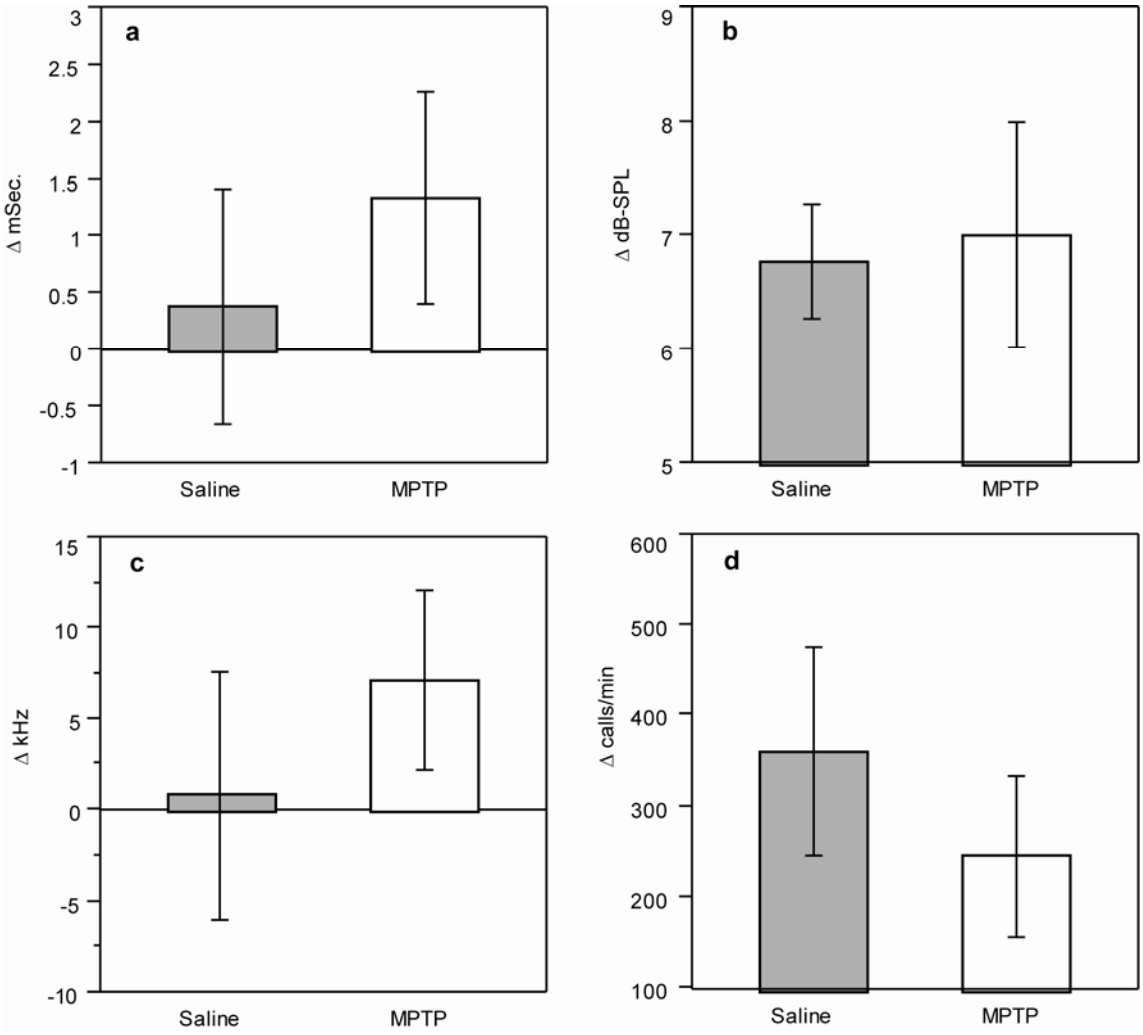

Fig 3.6. Effect of noise on echolocation call duration (a), amplitude (b), bandwidth (c), and call rate (d). Bats were flying after saline (grey bar) and MPTP (white bar) injection. There was no significant effect of MPTP on the effect of noise.

is clear the MPTP had a profound effect on vocalization. Bats treated with MPTP were unable to produce echolocation calls of a normal amplitude, duration, or frequency characteristics and produced calls at a rate lower than controls.

MPTP effect on call parameters

Amplitude

The decrease in call amplitude is likely due to a drop in sub-glottal pressure because of reduced neuronal input to the respiratory muscles to produce sufficient contractile force. A decrease in muscle tone is a known result of insufficient activation of the thalamus (Herrero et al., 2002), which occurs when DA levels in the striatum are abnormally low. One possibility is that the reduction of call amplitude is caused by a global reduction in muscle tone, and not vocalization specific. The fact that behavioral assays showed no impediments to movement, or other motor defects and that the bats were capable of normal locomotion, however, would argue against a system wide reduction in muscle tone. More likely is that the reduction in DA seems to have had a larger impact on the bats' ability to regulate vocal-respiratory musculature than the locomotor muscles, although it should be noted that I did not directly measure muscle force amplitudes for any other motor system.

Duration and frequency

The changes in call duration and frequency characteristics are the consequence of the reduction in call amplitude due to biomechanical constraints of the laryngeal apparatus. Previous evidence has shown that duration, F_{Start}, F_{End} and bandwidth are positively correlated with amplitude (Tressler and Smotherman, 2009). A drop in the

force of exhalation would prevent the bat from producing higher frequency calls, due to the mechanical properties of the larynx (Suthers and Fattu, 1973). What is unclear however, is if the shift from a more complex frequency modulated call to a structurally simple constant frequency call is due solely to this mechanical coupling, or if it represents an inability of the bat to manipulate the vocal apparatus in a complex way.

Surprisingly I found that bats receiving MPTP treatments could still fly, albeit in a straight line for only short distances. I did not challenge their ability to perform complex flight maneuvers. These simple flight experiments were sufficient to establish that the effects of MPTP on vocalization were completely abolished during flight (see fig 3.7 for representative call examples). One potential reason for this is that during flight, the bats utilize the force from the flight muscles to augment the respiratory muscles, coupling wing beat with call emission (Suthers et al., 1972). Flight muscles are coordinated by spinal pattern generators that appear to be independent of dopaminergic systems, although it seems likely that rigorous tests would reveal cognitive and coordination deficits in more challenging flight tasks. Never the less, it appears that with the aid of the flight musculature the bats were able to generate sufficient subglottic pressure to produce normal calls. If this is the case, than the changes in call structure

seen in stationary bats reflects the inability to generate sufficient sub-glottal pressure only in the stationary condition, and not an inability to control the rest of the vocal-motor apparatus.

Another possibility, however, is that echolocation behavior during flight is not initiated by the vocal-motor cortex, i.e. it is not strictly volitional. Based on the current hypothesized model of mammalian vocal motor control, the basal ganglia is only involved in the control of volitional vocalizations (Jürgens, 2009). If the echolocation behavior during flight were predominantly regulated by the midbrain vocal pattern generator, than alterations to the basal ganglia would not affect call structure or amplitude. The results of this experiment cannot adequately address whether flying abolished the effects of MPTP because of supplemental motor force from flight muscles or because during flight the cortical-striatal-thalamic loop is less important for the vocal control pathway.

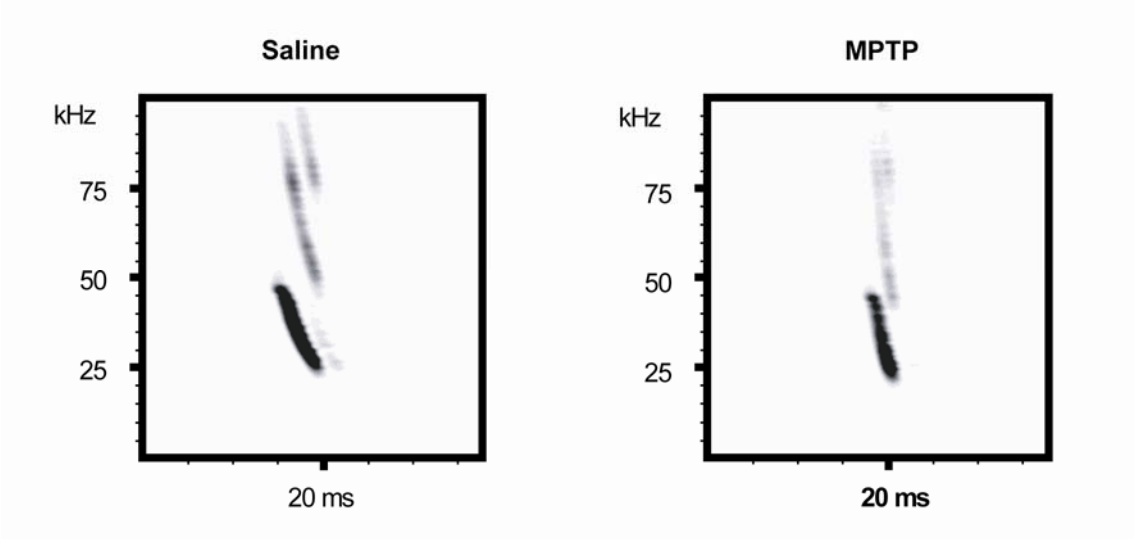

Fig 3.7. Representative examples of an echolocation call from a flying bat one hour after saline (Left) and MPTP (Right) injection.

Call rate

The reduction in call rate in the MPTP-bat can be explained by either a loss of motivation to call or an inability to initiate the vocal motor pathway. Unfortunately, it is impossible in the scope of this experiment to ascertain which is the case. The fact that MTPT did not alter the type of syllables uttered or the behaviors associated with vocalizing argues against a change in the motivational state, but does not rule out the possibility. Conversely, if it took longer in the MPTP bat for cortical activity to reach threshold due to reduced dopamine inputs this could also result in reduced instances and rates of vocalizations. This would be in keeping with the current model of BG motor control (Herrero et al., 2002; Nambu, 2004; Takakusaki et al., 2004). An experimental design that ensured motivation, such as vocal-operant conditioning, would be necessary to determine which condition was the case.

The decrease in all call parameters is consistent with results obtained from the ultrasonic vocalizations of rats. Ciucci et al (Ciucci et al., 2009) found that the amplitude and bandwidth of 50kHz ultrasonic call of male rats was significantly reduced by reduction of dopamine activity in the BG following unilateral lesions of the nigrostriatal pathway with the neurotoxin 6-OHDA. Also described was a reduction in trill-type frequency modulated calls for simpler constant frequency calls, a result that was also seen in the MPTP treated bats and reflected in the change in bandwidth (See fig 3.5). This suggests that the effect of decreased DA levels on vocalization is not a phenomenon unique to *Tadarida*, but may reflect a general feature of the mammalian vocal motor pathway.

MPTP effect on Lombard response

There is debate over whether human Parkinson's patients display a Lombard response or not. Adams and Lang (1992) reported that all 10 patients they tested displayed a Lombard response, suggesting auditory feedback may be a viable treatment for hypophonia (Adams and Lang, 1992). Ho et. al. showed, however, that background noise had no effect on voice volume in PD patients (Ho et al., 1999). This experiment represents the first in a non-human mammal to test if the BG is involved in control of the Lombard response. In stationary bats treated with MPTP, the presence of broadband noise had no effect on the amplitude of echolocation calls, apparently abolishing the Lombard response, supporting the conclusion that the basal ganglia are critical for the integration of auditory sensory cues into the volitional vocal motor commands. Unfortunately, because MPTP had such a large effect on the acoustic structure of echolocation calls in the absence of noise, it is not clear if the bats were capable of responding. Further testing will be necessary to determine if changes in dopamine activity can produce changes in the Lombard response without changes in baseline echolocation behavior.

The MPTP-Bat model of Parkinson's hypokinetic dysarthria

By examining the spectrogram of echolocation calls emitted by a bat treated with saline versus echolocation calls from the same bat after MPTP treatment (Fig 3.8), it is easy to see that MPTP had a profound effect on the structure of echolocation calls. These changes mirror many of the symptoms of parkinsonian hypokinetic dysarthria. One common symptom in human subjects is a reduced articulation with a breathy or

harsh voice. While difficult to quantify, this can be seen subjectively in the MPTP-bat. In addition to the timing and frequency shifts already discussed, echolocation calls from bats treated with MPTP display a non-distinct noise component to their echolocation. This distortion is unlike any recorded vocalization of the free-tailed bat (Bohn et al., 2008), and represents a degradation of vocal control. In addition, reduced bandwidth and amplitude (hypophonia) of vocalization is seen in both PD patients and the MPTP-bat. (See Table 3.3 for PD-MPTP comparison). Finally, it is often in the case in PD that the vocal deficits present themselves earlier than deficits in locomotion. Similarly, bats treated with the dose of MPTP used in this experiment developed vocal deficits without other motor disorders. In PD this is likely a function of the time course of disease progression while it is a function of dose dependency in the bat, but it is significant that it is possible to study the vocal pathology in the bat without confounding motor effects. Taken together, this data suggests that the MPTP-bat is a viable model for the study of Parkinson's like hypokinetic dysarthria.

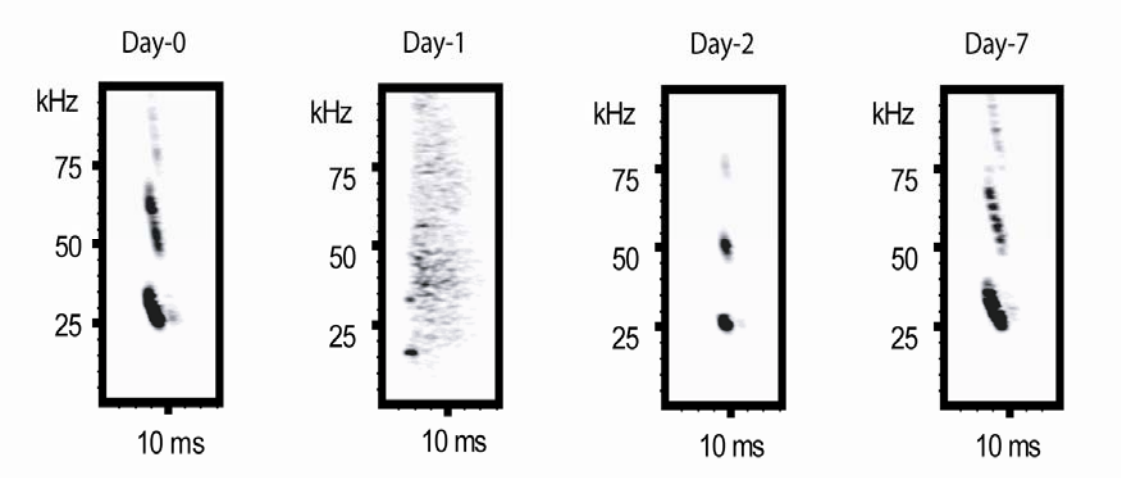

Fig 3.8. Representative examples of a typical echolocation call (Day-0), one hour after MPTP injection (Day-1), one day after (Day-2), and one week after (Day-7). The large amount of noise accompanying the echolocation call on Day-1 is indicative of the loss of motor control induced by MPTP. In addition, the reduction in bandwidth is easily visualized in both the Day-1 and Day-2 calls.

Table 3.3. A comparison of common features in Parkinsonian hypokinetic dysarthria and the homologous behaviors described in bats.

Comparison of Human and Bat Parkinsonian Vocal Disorders		
Features of Hypokinetic Dysarthria in Humans		Synonymous Vocal Deficit in MPTP-Treated Bats
Hypophonia		Significant decrease in echolocation call amplitude.
Reduced articulation		Presence of significant vocal artifacts indicating a lack of vocal control (see Fig 5)
Monotony of voice		Significant decrease in echolocation call bandwidth
Early Onset		Vocal deficits were present at doses of MPTP that did not induce other motor deficits

Conclusion

MPTP had a significant effect on the structure and loudness of echolocation pulses. Specifically, treatment with MPTP resulted in echolocation calls that were of lower amplitude, narrower bandwidth, shorter duration, and lower frequency (F_{Start}, F_{End}, and F_{Peak}) than those produced with saline treatment. These changes indicate that the dopaminergic system of the basal ganglia is critical for the correct production of volitional echolocation calls. Furthermore, MPTP nearly eliminated the Lombard response, supporting the hypothesis that the basal ganglia are involved in vocal-motor integration. Specifically, the results suggest that dopaminergic pathways play an important role in the generation and control of muscle force amplitudes essential for normal vocalizing. This is in stark contrast to current theories of vocal control that portray vocalizing as a product of brainstem pattern generators. These results mirror the symptoms of hypokinetic dysarthria in Parkinson's disease, and lay the groundwork for a MPTP-based animal model of Parkinsonian hypokinetic dysarthria.

CHAPTER IV

D1-TYPE RECEPTORS MODULATE VOCAL PLASTICITY

Introduction

The results of chapter III illustrate that bat vocal behaviors are very sensitive to chronic reductions of dopamine signaling in the bat basal ganglia. However, the use of MPTP to reduce dopamine release at synapses offers little insight into which basal ganglia circuits are most centrally involved in vocal control. Pathways through the basal ganglia have been broadly characterized as the direct (excitatory) and indirect (inhibitory) pathways, and these pathways are partly distinguishable based on dopamine receptor pharmacology. The direct pathway acts through what are known as D1-type dopamine receptors, and the indirect pathway acts via the D2-type dopamine receptors. It may be possible to show that drugs targeting one or both of these pathways preferentially affect the same vocal behaviors that were degraded by MPTP. If so, this would provide additional insight into the anatomical nature of the pathways controlling vocal plasticity in mammals. While it may generally be true that both pathways interact within the BG to control all volitional behaviors (Mink, 1996), one pathway may play a more active role in controlling vocalizing that the other.

The identification of pharmacological compounds that selectively target dopamine receptor subtypes has allowed for the specialized and localized role of these subtypes in specific aspects or stages of motor control to be examined in considerable detail. Previously, systemic injections of type-specific DA receptor ligands have been

used to successfully determine the role of specific DA receptors in motor control by altering receptor activity. For example D1 and D2 type ligands were used to determine the role of each receptor type in rat stereotyped grooming behavior (Berridge and Aldridge, 2000b, a) In the current experiment, systemic injections of the D1-type receptor agonist SKF82958 and antagonist SCH23390 will be used to test if the D1-type DA receptors mediate the sensorimotor feedback behavior (Lombard response) described in Chapter II and degraded in Chapter III.

Functionally, the basal ganglia is organized into 2 parallel pathways (Herrero et al., 2002). The direct pathway is predominantly modulated by the D1-type dopamine (DA) receptors, which facilitate neuronal transmission when activated. Because increased activity of direct pathway neurons in the putamen results in inhibition of the inhibitory connection from the substantia nigra pars reticulata (SNr) to the thalamus, the direct pathway is generally viewed as excitatory in nature. The indirect pathway, conversely, is considered an inhibitory pathway, as activation of indirect neurons in the striatum ultimately results in increased activity of inhibitory output neurons in the SNr and internal segment of the globus pallidus (GPi) to the thalamus. The D2-type DA receptors predominantly regulate the indirect pathway, with binding of DA to the D2-type receptors suppressing neuronal activity. The MPTP experiments decreased levels of DA in the striatum, which would have suppressed both the direct pathway and the indirect pathway, making it difficult to determine how each pathway is involved in regulating motor commands. It is hypothesized that by selectively blocking the direct, D1-mediated, pathway through the basal ganglia, I will reduce the inhibitory striatal

output to the GPi/SNr, which would in turn lead to increased inhibitory output from these output pathways. This increased inhibition is predicted to account for the primary symptoms of hypokinetic dysarthria, and it is therefore hypothesized that I can mimic the effects of the loss of dopamine with a D1-type DA receptor antagonist.

By only manipulating the D1-type receptors, it is hoped that the role of the direct pathway can be examined independently of indirect pathway activity. The receptor agonist SKF82958 and antagonist SCH23390 were selected for there high D1-type binding affinity (Gilmore et al., 1995; Bourne, 2001) and because they can be applied systemically and cross the blood-brain barrier. Early work with ultrasonic isolation calls of rat pups has shown that postnatal administration of SCH23390 resulted in increased call amplitude and duration (Cuomo et al., 1987), while another study on the 22kHz post-ejaculatory call in adults showed no effect on duration from either SKF82958 or SCH23390 (Cagiano et al., 1989). These conflicting reports may be due to the limbic nature of rat pup isolation calls. To correct for the likely possibility that the BG is not involved in non-volitional vocalization, this study will focus on volitional echolocation calls emitted by stationary free-tailed bats.

The basal ganglia is also known to play a role in the modulation of motor commands in response to sensory information (Groenewegen, 2003). What role it plays in audio-vocal integration, however, is unknown. Like other mammals free-tailed bats display a Lombard response, an increase in call amplitude in response to background noise. Increased voice amplitude is achieved by increased activation of the vocal respiratory muscles to build greater sub-glottal pressure. The basal ganglia are an

excellent candidate structure to coordinate the change in vocal-motor commands because of its established role in the control of muscle tone.

Because of the excitatory nature of the direct pathway, I hypothesize that increasing its activity with the D1-type receptor agonist SKF82958 will lead to an increase in echolocation call amplitude and duration, while decreasing its activity with the D1-type receptor antagonist SCH23390 will result in a decrease in call amplitude and duration. Further, it is predicted that activation of the D1-type receptors with an agonist will cause an increase in the Lombard response, while suppression with an antagonist will result in a decrease in the Lombard response.

Methods

Animal husbandry

Ten Mexican free-tailed bats, *Tadarida brasiliensis mexicana*, were caught wild from a year round roost on the campus of Texas A&M University and housed in the Texas A&M Department of Biology vivarium facility. Bats were kept on a phase-shifted 12/12 day/night cycle, with vivarium lights turning off at 12:00pm. The bat vivarium was a temperature and humidity controlled room that was large enough to allow the bats to fly freely. Bats were trained to feed themselves and had to fly daily to obtain food. The bats were fed a diet of mealworms supplemented with vitamins, minerals and essential fatty acids.

Acoustic stimuli

Acoustic stimuli consisting of broadband noise was generated digitally with Tucker-Davis Technology (TDT) system III hardware and the openEX software v5.4. The broadband noise was digitally filtered to present a total signal bandwidth spanning a range of 15 to 100 kHz, which covered the entire range of the two loudest harmonic components of *Tadarida brasiliensis'* echolocation pulses. Stimuli were played through a Sony amplifier (model # STR-DE598) driving a 2-speaker array composed of a Pioneer Ribbon Tweeter (ART-55D/301080) and a Pioneer Rifle Tweeter (ART-59F/301081), arranged to project directly at the experimental cage platform. Each speaker provided a flat (±3 dB) output at 85 dB SPL across the principal frequency range of interest, roughly 15 to 60 kHz. The bats' echolocation pulses ranged in intensity from 80 to 115 dB SPL at rest.

Audio recording design

All experiments were performed in an 8 meter long by 2 meter wide by 3 meter high flight tunnel lined with sound-absorbing 4-inch acoustic foam (Sonex ©,model UNX-4), with the lights off. Individual bats were placed in a 14x14x5cm wire mesh cage. Recordings were made using a Bruel & Kjaer Free-field ¼" microphone (Type 4939). The microphone was positioned 15cm from the bottom of the cage 9.75cm in from the cage corner. The placement of the microphone combined with the cage dimensions ensured that the experimental subject was facing the microphone from 12cm distance for the experiment. This method was used rather than head restraint to obtain the most natural echolocation behavior possible while still ensuring accurate measures of

pulse intensity. Recorded intensity of broadband noise was minimized by placing sound absorptive foam around the microphone on all sides except that facing the bat, which facilitated the digital extraction of echolocation pulses from the background noise. Incoming signals were digitized with a National Instruments DAQmx, NI PCI-6251 (200 kHz, 16 bit sample rate), and viewed with Avisoft Recorder v3.0. Pulse duration and intensity were analyzed using SASLab Pro v4.39 using the methods of Tressler and Smotherman (2009).

Pharmacological

Solid SCH23390 and SKF82958 salts were obtained from Sigma-Aldrich, St.Louis MO (cat. No. D054 & B135 respectively) dissolved in a 2% acetic acid solution and diluted with phosphate buffered saline. A 0.1ml intraperitoneal injection resulted in final dosages of 0.01 and 0.1ug/kg for SCH23390, 1.0, and 10.0 mg/kg for SKF82958.

Experimental procedure

Eight bats were selected at random from the captive colony. Each individual was used at most once in a 2 day period and no more than 3 times in a week. All experiments were conducted between 11:00 AM and 2:00 PM when the bats are normally most active in the vivarium. Bats were acclimatized to the experimental chamber before beginning each trial. First, each bat was injected with 0.1ml phosphate buffered saline and recorded for 30 minuets in the absence of broadband noise to provide a baseline measurement for comparison. In subsequent trials, each bat was recorded in the absence of broadband noise for 30 minuets after drug administration for each drug at both the high and low dose in order to determine both the time course and dose

dependency of any observed effect on pulse duration or intensity in the absence of acoustic interference. Pulses were grouped into 6, 5 minute bins, and the mean intensity duration, and total number of pulses was computed for each bat for each bin. Time period 1 refers to the first 5 min. post injection, time period 2 refers to the period between 5 and 10 min post injection, 3 between 10 and 15 min, 4 between 15 and 20 min, 5 between 20 and 25 min, and 6 between 25 and 30.

Individuals were then recorded echolocating in the presence of broadband noise for 10 minuets immediately following injection, for saline and SCH23390, and 10 minutes post injection for SKF82958. These time blocks corresponded to the periods when the respective drugs were found to have the greatest and most consistent effect on pulse parameters in the time course experiments. Again, both doses of both drugs were used to examine dose dependency effects. The mean pulse intensity and duration of all calls in the 10 minute period for each bat were used for analysis.

Statistical analysis

All statistical procedures were performed utilizing SAS-JMP v7.0.7. Analysis of the dopaminergic drugs on the Lombard response by MANOVA showed a significant overall effect of noise and drug ($P \leq 0.05$, $\alpha = 0.05$). Subsequent ANOVA analysis was performed to determine the significance of effect within parameters ($\alpha = 0.05$). Student's t-test pair-wise multiple comparison procedure ($\alpha = 0.05$) was used to determine significant differences between different treatments within a parameter if a significant effect of noise was found. Results are given as means ± S.D., unless stated otherwise.

Results

Time course of drug effects

Pulse number

Pulse number significantly changed over time (P=0.0492). Bats injected with saline emitted an average of 1391.444 +/- 235.295 pulses in time period 1, decreasing to 1177.0 +/- 285.446 then 732.222 +/- 252.896 in time periods 2 and 3 respectively, and finally reaching the lowest levels of 499.500 +/- 270.358 pulses in time period 4. Mean pulse number then increased slightly in time periods 5 and 6 to 607.571 +/- 305.227 and 943.833 +/- 306.746 pulses respectively.

Number of pulses per 5 min period did not tend to vary over the 30 min recording period for bats injected with 0.01 ug/kg of SCH23390. 778.778 +/- 275.279 pulses were emitted in time period 1, 1007.125 +/- 297.165 pulses in time period 2, 823.556 +/- 355.013 pulses in time period 3, 817.875 +/- 330.069 pulses in time period 4, 585.0 +/- 320.109 pulses in time period 5, and 573.833 +/- 261.098 pulses in time period 6.

I observed a tendency for pulse number to decrease over 30 min for bats injected with 0.1 ug/kg of SCH23390. A mean 496.0 +/- 127.874 pulses were emitted in time period 1 and 420.429 +/- 183.173 pulses in time period 2, increasing slightly to 596.857 +/- 235.074 pulses in time period 3, then decreasing dramatically to 164.667 +/- 74.104 pulses in time period 4 and 161.0 +/- 43.783 pulses in time period 5, before increasing slightly to 230.0 +/- 153.095 pulses in time period 6.

Bats injected with 1.0mg/kg SKF82958 displayed a temporary increase in echolocation pulse number before returning to near initial levels. Bats echolocated an average of 661.143 +/- 212.073 times in time period 1, increasing to 1251.286 +/- 260.427 times in time period 2, then 1418.286 +/- 251.746 times in time period 3 and 1493.286 +/- 221.997 times in time period 4, then decreasing to 1236.429 +/- 229.070 times in time period 5, and finally down to 1046.5 +/- 241.149 times in time period 6.

Bats injected with 10 mg/kg of SKF82958 showed an almost linear increase of pulse number over time. A mean 393.5 +/- 180.58 pulses were emitted in time period 1, increasing to 630.778 +/- 201.723 pulses in time period 2, then 783.889 +/- 199.426 pulses in time period 3, increasing further to 931.444 +/- 229.995 pulses in time period 4, and reaching a maximum number of echolocation pulses per 5 min period at 1144.125 +/- 241.753 pulses and 1149.875 +/- 230.401 pulses in time periods 5 and 6 respectively.

Duration

Pulse duration did not vary significantly across time (P=0.9465) regardless of treatment (P=0.1151). For bats injected with saline, the mean call duration for time period 1 was 3.279 +/- 0.313 ms, 3.222 +/- 0.272 ms in time period 2, 3.168 +/- 0.279 in time period 3, 2.983 +/-0.261 in time period 4, 3.035 +/- 0.391 in time period 5, and 3.135 +/- 0.410 in time period 6.

Mean pulse duration remained constant for 30 min for bats injected with 0.01 ug/kg and 0.1 ug/kg of SCH23390. At time period 1, the mean duration was 3.583 +/- 0.413 ms, 3.564 +/- 0.428 ms in time period 2, 3.529 +/- 0.299 ms in time period 3, 3.149 +/- 0.315 ms in time period 4, 3.438 +/- 0.249 ms in time period 5, and 3.412 +/-

0.359 ms in time period 6 for the lower dose of SCH23390 (Fig 2b). For the higher dose, the mean pulse duration in for time period 1 was 3.275 +/- 0.302 ms, 3.293 +/- 0.377 ms in time period 2, 3.860 +/- 0.631 ms in time period 3, 3.211 +/- 0.569 ms in time period 4, 3.474 +/- 0.443 ms in time period 5, and 3.333 +/- 0.475 ms in time period 6.

Mean pulse duration also remained constant for both doses of SKF82958. For the 1.0 mg/kg treatment the mean duration in time period 1 was 3.181 +/- 0.271 ms, 3.260 +/- 0.364 ms in time period 2, 3.418 +/- 0.310 ms in time period 3, 3.451 +/- 0.309 ms in time period 4, 3.470 +/- 0.288 ms in time period 5, and 3.464 +/- 0.166 ms in time period 6 (Fig 3b). For the 10 mg/kg treatment the mean duration in time period 1 was 3.238 +/- 0.397 ms, 3.540 +/- 0.400 ms in time period 2, 3.541 +/- 0.384 ms in time period 3, 3.565 +/- 0.334 ms in time period 4, 3.509 +/- 0.392 ms in time period 5, and 3.512 +/- 0.389 ms in time period 6.

Amplitude

Mean pulse amplitude did not vary significantly over time (P=0.3702) regardless of treatment (P=0.5439). Mean pulse amplitude for bats injected with saline was 114.870 ± 2.988dB-SPL in period 1, 114.556 ± 3.471dB-SPL in period 2, 113.925 ± 3.015dB-SPL in time period 3, 113.902 ± 3.655dB-SPL in time period 4, 113.376 ± 3.494dB-SPL in time period 5, and 114.193 ± 2.519dB-SPL in time period 6.

Mean pulse amplitude remained constant for bats treated with SCH23390. For the 0.01 ug/kg dose, mean pulse amplitude of 113.937 ± 3.076dB-SPL was observed in period 1, 113.100 ± 4.049dB-SPL in period 2, 114.769 ± 3.062dB-SPL in time period 3,

114.031 ± 3.743dB-SPL in time period 4, 113.375 ± 3147dB-SPL in time period 5, and 112.521 ± 4.178dB-SPL in time period 6 (Fig 2c). For the 1.0 ug/kg dose, mean pulse amplitude was 112.051 ± 3.773dB-SPL in period 1, 112.119 ± 3.828dB-SPL in period 2, 110.065 ± 5.168dB-SPL in time period 3, 109.968 ± 4.402dB-SPL in time period 4, 112.889 ± 5.386dB-SPL in time period 5, and 114.347 ± 6.809dB-SPL in time period 6.

Bats treated with 1.0 mg/kg and 10.0 mg/kg of SKF82958 also had a constant mean pulse amplitude over 30 min. For the 1.0 mg/kg treatment, mean pulse amplitude of 114.677 ± 4.688dB-SPL was observed in period 1, 113.328 ± 4.020dB-SPL in period 2, 114.544 ± 4.771dB-SPL in time period 3, 113.844 ± 3.723dB-SPL in time period 4, 113.612 ± 3.877dB-SPL in time period 5, and 112.655 ± 3.974dB-SPL in time period 6 (Fig 3c). For the 10.0 mg/kg dose, time period 1 had a mean pulse amplitude of 113.994 ± 5.210dB-SPL, 115.921 ± 5.629dB-SPL in time period 2, 115.101 ± 5.775dB-SPL in time period 3, 115.138 ± 5.852dB-SPL in time period 4, 115.143 ± 5132dB-SPL in time period 5, and 114.945 ± 4.957dB-SPL in time period 6.

Effect of D1 drugs on duration and amplitude in silence

Duration

The duration of echolocation pulses in silence was not significantly effected by

either SCH23390 or SKF82958 (P=0.0913). Mean echolocation pulse duration for bats injected with saline was 3.176 ± 0.896ms. 0.01 ug/kg and 0.1 ug/kg SCH23390 caused an insignificant increase in duration to 3.496 ± 1.084ms and 3.184 ± 0.933ms respectively. SKF82958 also caused an insignificant increase in duration to 3.516 ± 0.876ms and 3.581 ± 1.107ms for 1.0 mg/kg and 10.0 mg/kg doses respectively (Fig 4.1a).

Amplitude

The amplitude of echolocation pulses emitted in silence was not significantly altered by either D1 drug (P=0.3507). Mean pulse amplitude for bats treated with saline was 112.587 ± 2.773dB-SPL. SCH23390 caused a slight decrease in pulse amplitude, with the 0.01 ug/kg dose resulting in an amplitude of 111.766 ± 2.767dB-SPL and 110.244 ± 3.148dB-SPL for the 0.1 ug/kg dose. SKF82958 caused an increase to 114.218 ± 1.256dB-SPL for 1.0 mg/kg, and 113.178 ± 5.298dB-SPL for 10 mg/kg (Fig 4.1b). The mean duration and amplitude for calls uttered under each drug condition can be seen in table 4.1, the mean effect of drug on duration and amplitude for each drug can be seen in table 4.2.

Table 4.1. Mean call duration and amplitude of echolocation calls emitted in silence (noise off) or broadband noise (noise on). Bats were treated with either saline, or two different doses of the D1-type receptor agonist SKF82958, or the D1-type receptor antagonist SCH23390. Each value is the mean and standard deviation of the response of eight bats.

Drug	Dose	Noise	Duration (mSec)	S.D.	Amplitude (dB-(dB-SPL)	S.D.
Saline	0.1 ml	Off	3.176	0.896	112.587	2.773
Saline	0.1 ml	On	5.531	1.683	124.291	3.089
SCH23390	0.01 ug/kg	Off	3.496	1.084	111.766	2.767
SCH23390	0.01 ug/kg	On	5.797	1.559	123.010	5.462
SCH23390	0.1 ug/kg	Off	3.184	0.933	110.244	3.148
SCH23390	0.1 ug/kg	On	3.962	0.897	115.012	2.900
SKF82958	10 mg/kg	Off	3.581	1.107	113.178	5.298
SKF82958	10 mg/kg	On	4.524	1.180	118.081	3.916
SKF82958	1.0 mg/kg	Off	3.516	0.876	114.218	1.256
SKF82958	1.0 mg/kg	On	4.567	1.040	120.524	3.247

Table 4.2. **Effect of two doses of the D1-toye receptor agonist SKF82958 and antagonist SCH23390** on echolocation duration and amplitude in silence and broadband noise. Each value is the mean and standard deviation of the difference between drug and saline of eight bats.

Drug	Dose	Noise	Duration (Msec)	S.D.	Amplitude (dB-SPL)	S.D.
SCH23390	0.01 ug/kg	Off	0.320	1.009	-0.821	2.818
SCH23390	0.01 ug/kg	On	0.266	0.275	-1.281	4.977
SCH23390	0.1 ug/kg	Off	0.008	0.866	-2.343	2.524
SCH23390	0.1 ug/kg	On	-1.314	1.191	-8.935	3.525
SKF82958	1.0 mg/kg	Off	0.239	0.583	-0.168	3.228
SKF82958	1.0 mg/kg	On	-1.443	0.238	-5.234	2.148
SKF82958	10 mg/kg	Off	0.243	0.769	0.108	4.018
SKF82958	10 mg/kg	On	-1.417	1.169	-6.857	2.234

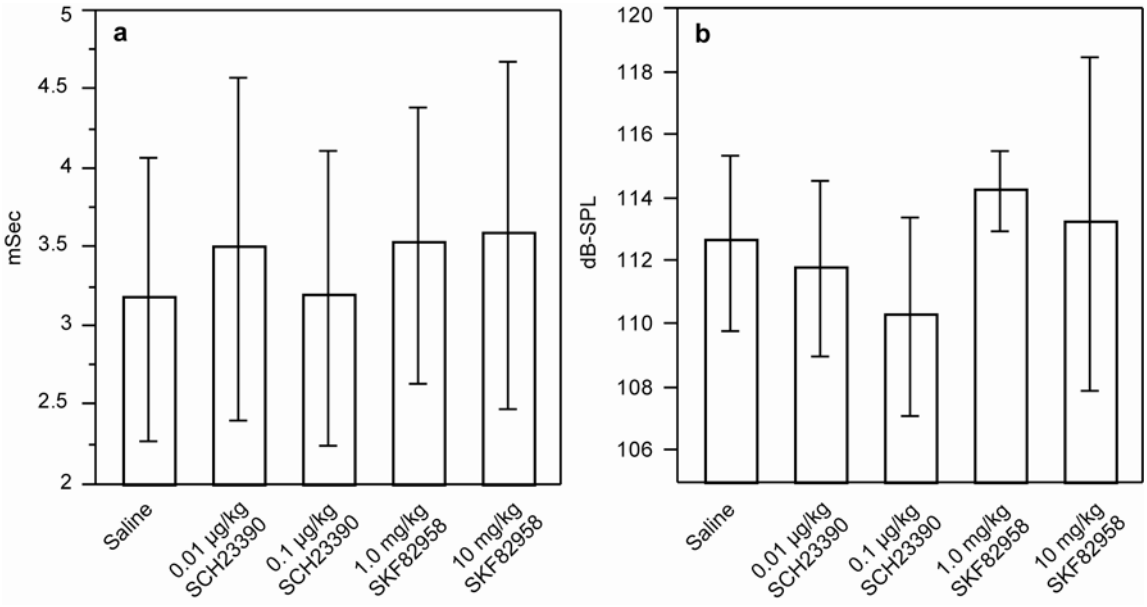

Fig 4.1. Effect of two doses of SCH23390 and SKF82958 on echolocation duration (a) and amplitude (b). There was no significant effect of either drug on either call parameter. Each bar represents the mean of duration and amplitude of 8 bats, error bars were constructed using 1 standard deviation.

Effect of D1 drugs on Lombard response

Duration

The amount that duration changed in response to noise was significantly effected by D1 drugs (P=0.0434). Specifically the 0.1 ug/kg SCH23390 caused a significant 1.496 ± 1.240ms decrease from the baseline saline response of increasing 5.531 ± 1.683ms in noise to only increasing 4.521 ± 0.744ms (α=0.05). The 0.01 ug/kg dose of SCH23390 and both doses of SKF82958 were not significantly different from saline (α=0.05). Bats treated with the low dose of SCH23390 increased their echolocation pulse duration in response to noise by 5.797 ± 1.559ms. Calls emitted in broadband noise by bats treated with 1.0 mg/kg and 10 mg/kg SKF82950 resulted in pulse duration increasing by 4.521 ± 0.744ms and 4.308 ± 1.132ms respectively (Fig 4.2,a).

Amplitude

D1 drugs significantly effected the increase in pulse amplitude in response to noise (P=0.0007). The high dose of both SCH23390 and SKF82958 both caused a significant decrease in the response to noise (α=0.05). When treated with saline, pulse amplitude increases in response to noise by 11.704 ± 3.657dB-SPL. The 0.1 ug/kg dose of SCH23390 caused a significant decrease in the response of noise of 8.200 ± 5.397dB-SPL to 3.504 ± 3.944dB-SPL. The 10 mg/kg dose of SKF82958 also caused a significant decrease in the response to noise, the change in pulse amplitude decreased by 6.662 ± 2.787dB-SPL to 5.324 ± 3.222dB-SPL (Fig 4.2,b).

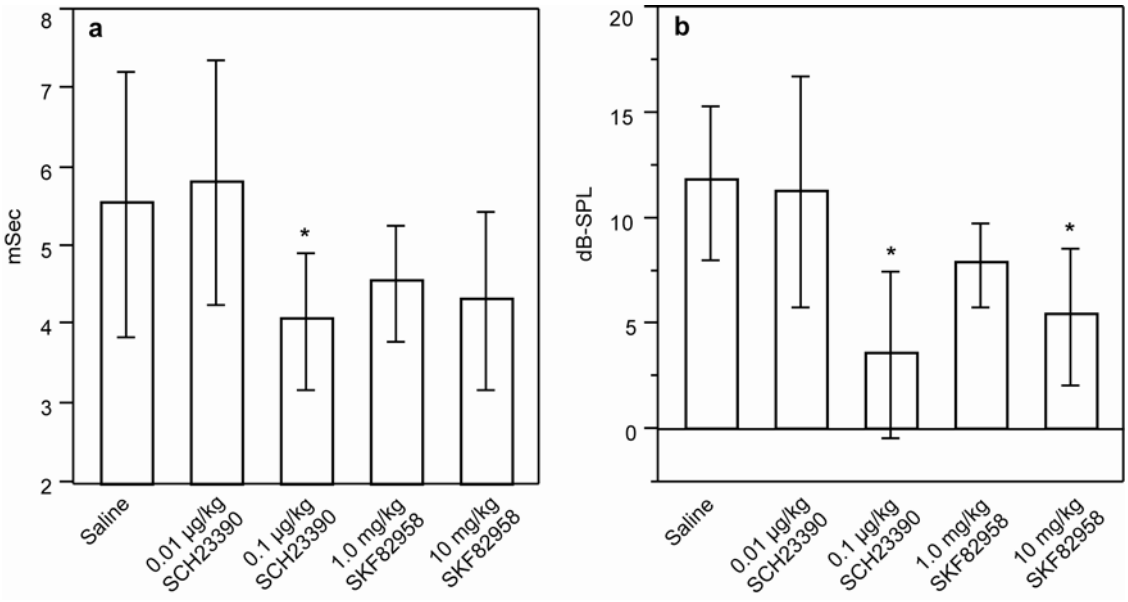

Fig. 4.2. The effect of noise on echolocation call duration (a) and amplitude (b) in bats treated with either saline, or 2 doses of SCH23390 or SKF82958. Each bar represents the mean response to noise of 8 bats, error bars are constructed with 1 standard deviation. Both drugs caused a significant change in the response to noise. Bars marked with a asterisk are significantly different from saline (α=0.05).

Discussion

The D1-type receptor agonist and antagonist SKF82958 and SCH23390 did not have a significant effect on the acoustic parameters of echolocation calls emitted in silence for any dose tested, indicating that the D1-type receptors are not actively involved in the control of echolocation calls in silence. Furthermore, neither drug affected the number of calls emitted, indicating that there was no effect on motivation to vocalize. Similarly, in was previously reported that the duration of the rat's postejaculatory 22kHz ultrasonic vocalization was unaffected by systemic administration of SCH23390 or SKF82958 (Cagiano et al., 1989). Conversely, it was reported that SCH23390 caused rat pup ultrasonic vocalizations to increase in both amplitude and duration (Cuomo et al., 1987), and it was reported that D1 receptor agonists caused a reduction in the number of isolation calls emitted (Dastur et al., 1999). This may indicate that isolation calls are regulated by a different pathway than other vocalizations, or that there are developmental changes that occur in the dopaminergic system between the pup and adult stage.

Both the D1 agonist and antagonist significantly affected the Lombard response at the highest dose tested. As expected the D1 antagonist significantly decreased the magnitude of the change in call amplitude induced by background noise, i.e. reduced the Lombard response. Surprisingly, the higher dose of the agonist also causes a significant reduction in the Lombard response. That either compound causes a change in the Lombard response indicates that the D1-type synapses are involved in the modulation of the vocal response to noise. Unlike the bats treated with MPTP in Chapter III that may

have been incapable of increasing call volume; because calls emitted in silence were normal, it is unlikely that the bats were not capable of generating the respiratory force needed to increase call amplitude. This provides additional support to the hypothesis that the D1-type synapses are involved in vocal-motor integration, not just regulating respiratory muscle tone.

The fact that SCH23390 and SKF82958 both have the same effect on the Lombard response indicated that the D1-type receptors modulate the response to noise in a complex way, likely involving interaction with another control mechanism. Given the preponderance of D1-type receptors associated with the direct pathway in the BG, it is reasonable to hypothesis that the direct and indirect pathways work in concert to regulate integration of acoustic stimuli into vocal motor commands. Balance between the action of the direct and indirect pathway is the current hypothesis of BG control of motor programs (Grillner et al., 2005). As was seen in this experiment, forcing the system out of balance in either direction might result in inappropriate motor patterns being selected or modulated incorrectly.

D1-type synapses, and by extension the direct pathway of the basal ganglia, appear to be critically involved in the modulation of vocal-motor adaptations triggered by background noise. The nature of that control is clearly complex, and likely involves the combined action of the indirect pathway. Confirmation of this hypothesis will require additional experimentation on the D2-type receptors specifically. The results of this experiment provide evidence to support the hypothesis that the basal ganglia are

involved in the integration of auditory stimulus into vocal motor commands and may provide a site for control of vocal plasticity.

CHAPTER V

CONCLUSION

The goal of this thesis was to improve our understanding of the functional organization of vocal-motor circuits in a mammal that exhibits exceptional vocal plasticity. Vocal plasticity is the hallmark of human speech and language, and although no other animal displays a comparable behavior, animals such as songbirds and echolocating bats can provide useful insight into some of the mechanisms that make speech possible. In this dissertation, I have attempted to demonstrate that echolocating bats can serve as useful models of how sensory feedback drives changes in vocal production. Although much work has been done on elucidating the basic features of the mammalian vocal pathways in primates (Jürgens, 2002a), cats and bats(Schuller and Radtke-Schuller, 1990; Brainard and Doupe, 2000), current models do not account for any form of vocal plasticity. It has been widely hypothesized that in humans and songbirds this plasticity derives from the neural circuitry of the basal ganglia (Brainard and Doupe, 2000). I therefore set out to find experimental evidence of basal ganglia involvement in bat vocal plasticity. The results have provided three separate lines of evidence supporting basal ganglia involvement in vocal control: 1) behavioral studies showed that the vocal responses to auditory feedback were dependent on context. One of the primary functions of the basal ganglia is that it is believed to serve as a contextual gate for sensory feedback effects on motor control (Aldridge et al., 2004; Kao and Brainard, 2006). 2) Pathological disruption of dopamine signaling using a method that mimics basal ganglia dysfunction in humans caused a degradation of vocal control in

bats that closely resembled the symptoms of hypokinetic dysarthria in humans. These symptoms in humans are widely presumed to derive from reductions in dopamine signaling in the basal ganglia. 3) Selective pharmacological blockade of dopamine synapses had a significant effect on the bats ability to make normal vocal responses to noise. Collectively these results strongly support the conclusion that the basal ganglia are involved in vocal modulation, and to a lesser extent vocal initiation, in mammals. Below I will review how each of these experiments provided more details about vocal control.

As seen in Chapter II, *Tadarida brasiliensis* displays a complex, context dependent response to acoustic stimuli. In narrow band noise, the bats displayed a stimulus-frequency dependent shift in echolocation call frequency that occurred without changes in call amplitude, i.e. the jamming avoidance response. In broadband noise the bats respond by increasing call amplitude linearly with background noise amplitude, a Lombard response. Both examples of vocal plasticity are robust and consistent across individuals, and provide a valuable tool for studying the underlying neurobiology of vocal-motor control. The results show that these bats are capable of switching between two distinctly different vocal responses dependent on the acoustic nature of the stimulus. Further, how the bats respond to a given kind of stimulus is dependent on additional sensory information. More simplistically, the magnitude of the Lombard response is dependent on the intensity of background noise, more complex; the frequency of band-limited noise that best evokes a JAR is dependent on the spectral characteristics of the emitted pulse, especially the F_{peak}. This level of responsiveness to different stimuli can

provide a powerful tool for examining the neurocircuitry that underlies the vocal plasticity that is crucial for human speech.

Reduced levels of dopamine resulted in echolocation calls that were significantly altered (Chapter III). These MPTP induced changes were characterized by a reduction in echolocation call rate, amplitude, bandwidth, and duration, indicating that the basal ganglia plays a significant roll in the control of volitional vocalizations. MPTP also caused a complete loss of the Lombard response in stationary bats, supporting the hypothesis that the basal ganglia are important for sensory-motor integration and vocal plasticity. The severe effects of MPTP on echolocation call amplitude and structure made the effect on the Lombard response difficult to interpret.

The observed vocal deficits in MPTP treated bats mirrored the vocal deficits recorded in Parkinson's patients. Specifically, the bats clearly manifested reduced call amplitude and bandwidth, conditions synonymous with hypophonia and monotony of voice in humans. PD patients will often manifest lack of articulation (Sapir et al., 2008). While it is difficult to quantify for this experiment, the MPTP-bats clearly displayed a loss of vocal-motor control, which manifested as emission of an unregulated noise accompanying echolocation call emission. Because of the similarities in vocal pathology, a MPTP-bat model of Parkinson's disease could be invaluable in the evaluation of therapeutic techniques for PD. The bat would provide for the first time an animal model for the study of parkinsonian vocal deficits.

The specific D1-type receptor agonist and antagonist SKF82958 and SHC23390 provided additional evidence for the mechanism of basal ganglia control of vocal plasticity (Chapter VI). Both SKF82958 and SCH23390 significantly reduced the Lombard response without affecting the structure of echolocation calls in silence, indicating that the D1-type receptors, most likely those in the basal ganglia, are involved in the modulation of auditory induced vocal plasticity. Further, because both the agonist and antagonist produced the same response, it is clear that modulation of the vocal-motor program involves a more complex circuit, likely involving the indirect pathway.

The evidence that perturbation of the D1-type receptors in either direction produced a suppression of behavior has implications for the development of drug therapies targeting dopaminergic systems. Specific receptor agonists have been used to augment L-dopa treatment in PD for years (Stocchi, 2009), but often with unexpected side effect. A biphasic dose response of dopamine has been previously described as applying to numerous endpoints (Calabrese, 2001), and it is not surprising that vocalization would be among them. If a majority of the basal ganglia interactions works via a balance between these competing pathways, then understanding their interaction is a key first step in designing treatments that are more effective. The BG control of vocal plasticity in response to noise provides an excellent background for further research, as it is robust, predictable, and can be perturbed without harming the animal, allowing for multiple preparations on the same subject.

It should be mentioned that while the conclusions above represent the most parsimonious interpretations of the experiments, they are not the only explanations.

Parkinson's disease is traditionally thought to be a disorder of the basal ganglia only, but recent evidence has shown that several other brain regions, such as the brainstem, neocortex, amygdala, and hippocampus are effected as well (Braak and Braak, 2000; Braak et al., 2004). The results of MPTP treatment could arise from changes in activity of any of these regions. For example, there is some evidence that somatosensory pathways mediated by the locus coeruleus are dampened and become inefficient due to the loss of noradrenergic neurons in the brainstem (Hammer and Barlow). The loss of somatosensory feedback is particularly debilitating to the speech motor pathways owing to the need to track respiratory status and the positions of the multiple laryngeal articulating cartilages. Loss of somatosensory feedback could underlie poor articulation in PD speech disorders. Furthermore, cognitive deficits arising from the loss of dopamine in the frontal cortex and hippocampus are very likely to degrade speech fluency levels, but little work has been done to characterize these effects in any animal.

The results of Chapter IV are somewhat confounded by the presence of D1-type receptors in parts of the brain other than the direct pathway basal ganglia, such as the cortex, cerebellum, hippocampus, amygdala, and cingulate cortex (Hall et al., 1994), as well as the periphery. While these structures are not known to modulate motor plasticity in any other system, and the level of D1-type receptor expression is significantly lower than in the basal ganglia, it is possible that the D1 ligands are acting at other locations, and that the direct pathway is not involved. Additional experimentation, utilizing techniques such as focal injection, that eliminate other areas as sites of possible action would need to be conducted to address these possibilities.

In the currently hypothesized mammalian vocal motor pathway, volitional vocalizations are initiated in the motor cortex and executed in the mid and hindbrain vocal pattern generators. Until recently, the basal ganglia were thought to only be involved in controlling vocalization in humans and songbirds. This study would suggest, however, that the basal ganglia are likely important in any mammal that produces volitional or learned vocalizations or displays vocal plasticity. These studies indicate that the basal ganglia are a necessary part of the forebrain vocal motor pathway. Further, the basal ganglia are involved in two aspects of vocal control. 1) It regulates muscle tone in muscles required for vocalization, and 2) it is necessary for the proper integration and response to acoustic sensory stimuli in the vocal-motor pathways. Finally, as in other instances of motor control, proper regulation of vocal plasticity requires a coordinated interaction between the D1- and D2-type receptors.

It appears that, in general, dopamine is involved in regulating complexity of vocalization, either by directly influencing call shape or by influencing call selection. In nature, bats must respond to a continually changing environment and must select appropriate vocal behaviors as the situations change. Beyond the changes in acoustic environment examined in these studies, dopamine may play a greater roll in responding to changes in context. It is reasonable to hypothesize that increasing levels of dopamine helps prompts the switch from the bats more stereotyped and structurally simple calls, like the cf search calls, to more dynamic and complex calls. Given dopamine and the basal ganglia's roll in reward anticipation, this could help drive the use of dynamic calls during group hunting bouts and as a mechanism for promoting the complex

communication sequences used in mate and territory defense. It is likely that dopamine is involved more than regulating muscle tone, but also in regulating motor commands in response to a changing environment.

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